STUDIES ON THE INTERACTION OF ROOT-KNOT NEMATODE, MELOIDOGYNE WITH ROOT-ROT FUNGUS, RHIZOCTONIA ON COWPEA

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ATUL NAMDEO
M. Sc., M. Phil.

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DR. V.P.VARSHNEY
Head

DEPARTMENT OF BOTANY
BIPIN BIHARI (P.G.)COLLEGE
JHANSI-284001 (U.P.)

This is to certify that Mr. Atul Namdeo has worked in this Department as a Project Assistant under a research project sponsored by University Grants Commission, New Delhi under my supervision and guidance. His work "STUDIES ON THE INTERACTION OF ROOT-KNOT NEMATODE, MELOIDOGYNE WITH ROOT-ROT FUNGUS, RHIZOCTONIA ON COWPEA" is upto date and original. He is allowed to submit his thesis for the consideration of award of the degree of Doctor of Philosophy in Botany.

He has put more than 200 days of attendance in the Department as required by the Bundelkhand University ordinance under 7 (ii).

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V.P.Varshney
(Dr. V.P.Varshney)

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ATUL NAMDEO

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INTRODUCTION AND REVIEW OF LITERATURE

Plant parasitic nematodes, though microscopic, are responsible for causing considerable economic losses. It has now been firmly established that plant parasitic nematodes are bane in crop production. In a recent report Feldmesser et al. (1971) estimated annual losses due to nematodes to the tune of $ 1,038,374,300 for field crops; $ 225,145,900 for fruits and nuts crops; $ 266,989,100 for vegetable crops and $ 59,817,634 for ornamental crops. In India, Van Berkum and Seshadri (1970) reported annual losses due to "Ear-cockle" disease caused by Anguina tritici (Steinbuch, 1799) Chitwood, 1935 on wheat amounting to 10 million dollars; due to "Molya" disease caused by Heterodera avenae Wollenweber, 1924 on barley to 8 million dollars and due to Pratylenchus coffeae (Zimmermann, 1898) Goodey, 1951 on coffeae to 3 million dollars.

In nature root of plants are exposed to a variety of microorganisms which form the common components of soil biosphere forming a variety of relationship including synergistic. In such multipathogenic conditions the losses to plants are much more than in monopathogenic conditions. The various aspects of multipathogenic effects on plants have been reviewed by Pitcher (1963, 65); Powell (1963, 1971a & b, 1979); Miller (1965); Bergeson (1972) and Khan (1981). It has been observed that presence of more than one kind of microorganisms modifies disease expression, and at times breaks even the resistance of plants (Holdman and Graham, 1954;

Sasser et al. 1955; Jenkins and Coursen, 1957; Thomason et al. 1959 and Devis and Jenkins, 1963).

Plant parasitic nematodes are often involved in disease complexes with fungi responsible for wilts, root-rots and various seedling diseases.

I. NEMATODE - FUNGUS WILT DISEASE COMPLEXES

In most of the nematode-wilt causing fungal disease interactions, Fusarium or Verticillium are involved. In 1892 Atkinson for the first time reported that root-knot and Fusarium caused distinct diseases of cotton, however, far more damage was caused when both were present in the soil. Further, cultivar resistant to Fusarium became susceptible if root-knot was present in the field. Since then many reports have been published involving Fusarium or Verticillium and root-knot nematode on a variety of crops.

A synergistic interaction between F. oxysporum (Woll.) Snyder & Hansen and M. incognita on cotton has also been reported by Perry (1961, 1963). Minton and Minton (1963) working with root-knot and Fusarium complex on cotton reported that the fungus colonized the tissues damaged by root-knot, M. incognita acrita. Holdman and Graham (1954) found that the presence of even sting nematode, Belonolaimus gracilis Steiner, 1949 resulted in breaking the resistance of cotton cultivar resistant to Fusarium wilt. According to Michell and Powell (1972) simultaneous inoculation with

Pratylenchus brachyurus (Godfrey, 1929) Filipjev & Stekhoven, 1941 and F. oxysporum f. vasinfectum (Atk.) Snyder & Hansen caused higher percentage of wilting in Fusarium susceptible cotton plants as compared to those in which nematode preceded the fungus by two week or when the fungus alone was used. Schanmugam et al. (1977) observed that in cotton plants wilting was more severe and disease symptom developed earlier when both Hoplolaimus seinhorsti and F. oxysporum f.vasinfectum were present than either of the two organisms alone.

Melendez and Powell (1965, 1967) pointed out that galled tissues of both resistant and susceptible varieties of flue-cured tobacco were more favourable sites for colonization of F. oxysporum f. nicotianae Johnson. Porter and Powell (1967) found that simultaneous inoculation of plants with F. oxysporum f. nicotianae and M. incognita or M. arenaria (Neel, 1889) Chitwood, 1949 or M. javanica (Treub, 1885) Chitwood, 1949, caused less damage to tobacco seedlings as compared to those in which nematode inoculation preceded the fungal inoculation by 2-4 week. Powell and Batten (1969) reported that association of M. incognita and Fusarium on tobacco allowed Alternaria tenuis Nees to develop on diseased plants. Noguera (1977) reported that M. incognita predisposed tobacco plants to the attack of F. oxysporum f. sp. batatus.

Young (1939); Harrison and Young (1941) reported that root-knot nematode greatly decreased the resistance of many

tomato varieties to Fusarium wilt. Jenkins and Coursen (1957) could induce 100% wilting in Fusarium wilt resistant tomato variety 'Chesapeake' in the presence of M. incognita acrita, while only 60% in the presence of M. hapla Chitwood, 1949. Binder and Hutchinson (1959), on the otherhand, were not able to get positive results. Bowman and Bloom (1966) demonstrated indirect relationship of M. incognita to the breaking of resistance to Fusarium wilt in tomato and they were of the opinion that M. incognita appeared to change the physiology of the entire plant and thereby making it more susceptible to Fusarium wilt. Kawamura and Hirano (1967, 1968) reported that simultaneous inoculation with M. incognita and F. oxysporum f. lycopersici caused more severe damage to the tomato seedlings. Webster (1975) also reported that Fusarium wilt of tomato was more severe in the presence of M. incognita whereas Liburd and Mai (1976) observed that Fusarium wilt in tomato was not only severe in presence of root-knot nematode but also developed much earlier. Miller (1975), on the other hand, observed that severity of wilt in tomato was reduced in the presence of Heterodera tabacum Lownsbery and Lownsbery, 1954. A synergistic interaction between M. incognita and F. oxysporum f. sp. lycopersici on tomato was also reported by Carter et al. (1977).

Thomason (1958) reported that wilt caused by F. oxysporum f. tracheiphilum (E.F.Sm.) Snyder & Hansen increased both in susceptible variety 'Chino 3' and in

resistant variety 'Grant' in the presence of M. javanica. Thomason et al. (1959) observed that the presence of M. javanica was not only able to break the resistance of 'Grant' variety but increased the wilt to such an extent that wilt was more than on Fusarium susceptible variety 'Chino 3'.

Ross (1965) observed that Fusarium oxysporum caused more damage to soybean in the presence of Heterodera glycines Ichinohe, 1952 as compared to M. incognita. Goswami and Agarwal (1978), while studying the interaction of four species of Fusarium with M. incognita on soybean observed an antagonistic interaction with F. oxysporum and F. solani and a synergistic interaction with F. graminearum and F. equsiti.

McGuire et al. (1958) observed that in alfalfa wilting percentage caused by F. oxysporum f. vasinfectum increased when inoculated with either of the four species of root-knot namely M. hapla, M. javanica, M. incognita or M. arenaria. Griffin and Thyr (1978) observed that F.oxysporum caused marked reduction in growth in lucerne variety susceptible to M. hapla and not in resistant variety on inoculation 30 days after nematode inoculation.

Davis and Jenkins (1963) observed that the presence of M. incognita acrita/M. hapla broke the resistance of pea variety 'Alaska' to F. oxysporum f. pisi race 1. Increase in wilting caused by F. oxysporum f. pisi race 2 in the presence

of Pratylenchus penetrans (Cobb, 1917) Chitwood and Oteifa, 1952 was reported by Seinhorst and Kuniyasu (1971).

The presence of Radopholus similis (Cobb, 1893) Thorne, 1949 in the soil doubled the wilt percent in banana caused by F. oxysporum f. cubens (E.F.S.) Snyder & Hansen (Newhall, 1958). Van Gundy and Peter (1963) observed greater reduction in growth of citrus seedlings when inoculated with citrus nematode, Tylenchulus semipenetrans Cobb, 1913 and Fusarium solani (Mart) Snyder & Hansen than with either of the two alone.

Gill (1958) observed severe wilting of mimosa seedlings when they were inoculated with F. oxysporum f. perniciosum (Hepting) Toole and either M. incognita or M. javanica. Similar results were observed by Sumner and Johnson (1973); Khan and Saxena (1969), while studying the interaction of M. incognita with F. oxysporum f. sp. nivem Snyder & Hansen on watermelon and with F. oxysporum var. lycopersici on okra respectively.

Singh et al. (1981) observed an increase in Fusarium wilt in presence of M. incognita in Phaseolus vulgaris. Chahal and Chabra (1985) while working with pea cultivars observed that the combined inoculation of M. incognita and F. equiseti caused significant reduction in growth as compared to by either of the pathogen alone.

Thakar et al. (1986) reported that Cicer arietinum line ICC-12275 show no wilt when inoculated with F. oxysporum f. ciceri but in presence of M. incognita this fungus caused 50% mortality of the plants. Sakhuja and Sethi (1986) studied the effect of M. javanica, F. solani and R. bataticola on Arechis hypogea in twelve different combinations. It was found that both fungi produced galling significantly in all treatments except where F. solani succeeded nematode after a week. The maximum reduction in nematode multiplication was reported where one or both fungi were inoculated simultaneously with the nematode. The fungus R. bataticola exhibited greater antagonistic activity as compared to F. solani.

Upadhyay and Dwivedi (1987) studied the effect of inoculation of M. javanica alone and in combination with F. oxysporum f. ciceri on chickpea seedlings var. 'Arodhi' and found that plants inoculated with fungus alone showed slight wilting but wilting was maximum and rapid in plants where nematode inoculation preceded the fungus.

Kumar et al. (1988) while studying the disease complex in chickpea involving M. incognita and F. oxysporum observed that though both the pathogens individually cause damage to the plants but in biopathogenic conditions, highest damage was observed when both the pathogens were inoculated simultaneously followed by the treatment in which the nematode preceded the fungus and then by the one in which the fungus preceded the nematode.

Hasan (1989) observed a significant increase in wilting when Cajanus cajan plants were inoculated with Heterodera cajani three weeks prior to the fungus F. udum.

Synergistic relationships of Verticillium were observed with a variety of nematodes, as for example V. alboatrum Reinke & Berthold with Pratylenchus penetrans on eggplant (McKeen and Mountain, 1960), tomato (Conroy et al. 1972), potato (Morsink and Rich, 1968; Burpee and Bloom, 1974), Peppermint (Bergeson, 1963), pepper (Olthof and Reyes, 1969) and Impatiens (Muller, 1972); with Tylenchorhynchus capitatus Allen, 1955 and M. incognita (Overman and Jones, 1970), Trichodorus christiei Allen, 1957 (Conroy and Green, 1974) and Heterodera tabacum (Miller, 1975) on tomato; with M. hapla on potato (Jacobsen et al., 1979); V. dahliae Kleb. with P. penetrans on tomato (Mountain and McKeen, 1962); with P. minyus Sher and Allen, 1953 on peppermint (Faulkner and Skotland, 1965) and with M. hapla or P. penetrans or Tylenchorhynchus claytoni on cherry (Ndubizu, 1977).

II. NEMATODE - FUNGUS ROOT-ROT COMPLEXES

Sasser et al. (1955) observed that the presence of root-knot nematode reduced the resistance in tobacco varieties to black shank fungus Phytophthora parasitica f. nicotinae (Bredade Hann) Tucker. Powell and Batten (1967) and Melendez and Powell (1969) found that tobacco seedlings and even mature plants were least affected by Rhizoctonia solani Kuhn,

Pythium ultimum Trow and *Trichoderma harzianum* Rifai, however, they caused extensive damage even to mature plants which were already infected with *M. incognita*. However, simultaneous inoculation with the above fungi and root-knot resulted in little damage. Again Melendez and Powell (1970) reported that *M. incognita* predisposed flue-cured tobacco roots to *Pythium ultimum* and damage to roots was more when the nematode preceded the fungus. Powell *et al.* (1971) observed an interesting disease complex between soil inhabiting genera of fungi such as *Pythium*, *Curvularia*, *Botrytis*, *Aspergillus*, *Penicillium* and *Trichoderma* and root-knot *M. incognita* on flue-cured tobacco cultivar C 316 and found that severe necrosis on tobacco roots occurred when the nematode preceded the fungi by several weeks but none of the fungi induced disease in the absence of *M. incognita*.

Dunn and Hughes (1964) and Dunn (1968, 1970) found more reduction in growth of tomato when *Heterodera rostochiensis* entered the roots before *R. solani* and *Colletotrichum atramentarium* (Berk. and Br.) Taub. than when fungus preceded the nematode or when the two pathogens entered simultaneously. A disease complex between root-knot nematode *M. incognita* and *R. solani* on tomato (Sethi, 1966) and on okra and tomato (Golden and Van Gundy, 1975) was also reported. Goswami *et al.* (1975), while studying the interaction of *M. javanica* and *Rhizoctonia bataticola* on tomato, reported that nematode inoculation 3 week prior to *R. bataticola* resulted in 33.3

percent wilting against 6.7, 13.3 and 6.7 on inoculation with fungus alone or with both simultaneously or with fungus 3 week prior to nematode respectively. Roy (1977) observed that prior inoculation of Heterodera rostochiensis to R. solani or Colletotrichum coccodes caused greater growth reduction and incidence of disease in tomato cultivar 'Ailsa Craiz' than when the fungi preceded the nematode.

Goswami et al. (1970) observed that 6 and 25 percent plants wilted when bringal seedlings were inoculated with Sclerothium rolfsii Sacc. alone and with both M. incognita and fungus respectively. The root-rot of eggplants caused by either R. solani, Pythium spp. or Colletotrichum atramentarium was aggravated in the presence of M. incognita and the damage to eggplants was more when either of the three fungi was inoculated simultaneously with M. incognita (Azam, 1975). Sinha et al. (1977) observed an antagonistic interaction between M. incognita and Ozonium texanum var. parasiticum as both the pathogens were independently harmful to brinjal plants but their harmful effect on plant growth was reduced when the nematode and fungus were present together.

Hedrick and Southards (1976) observed heavy damage to soybean by Cylindrocladium crotolariae in the presence of M. incognita. More damage occurred when nematode preceded the fungus. Adeniji (1977) reported more severe disease development in soybean cultivars susceptible to Phytophthora

megasperma Drechs var. sojae Hild (Pms) in presence of Heterodera glycines than when the fungus (Pms) alone was present. Raut and Sethi (1981), while studying the interaction of M. incognita with Rhizoctonia bataticola and Fusarium solani on soybean, observed that the adverse effect of nematode was mitigated to a great extent when either of the fungi preceded the nematode inoculation but the prior inoculation of M. incognita to R. bataticola was synergistic in reducing the top growth.

Grainger and Clark (1963) observed that even moderate infestation with R. solani and Heterodera rostochiensis caused considerable decrease in potato yield.

According to Price and Schneider (1965), Polychronopoulos et al. (1969) and Polychronopoulos (1970) mixed infection of Heterodera schachtii Schmidt, 1871 and R. Solani considerably promoted root and seedling damage in sugarbeet. Jorgenson (1970), on the otherhand, observed that mixed infection of sugarbeet with H. schachtii and F. oxysporum caused less damage to sugarbeets as compared to caused by nematode alone.

Apt and Koike (1962) reported that the presence of M. incognita acrita and root-rot fungus Pythium graminicola Subram. reduced top growth of sugarcane but not the root growth while Santo and Holtzmann (1970) reported that simultaneous inoculation with Pratylenchus zeae Graham, 1951 and Pythium graminicola reduced both top and root growth more,

than that with either of the pathogens alone. The damage aggravated when nematode inoculation preceded the fungus.

O'Bannon et al. (1967) reported an increase in root decay by Fusarium spp. in lemon if Tylenchulus semipenetrans was also associated.

Mountain and Bendict (1956) reported that Pratylenchus minyus and Rhizoctonia solani combination caused two fold reduction in growth of winter wheat. Kisiel et al. (1969) noticed that Tylenchus agricola de Man, 1884 Filipjev, 1934 also contributed towards the increase in root-rot of corn caused by Fusarium roseum (Link.) Snyder & Hansen. A synergistic interaction between M. incognita and Fusarium moniliforme Scheldon on maize has been reported by Palmer and Mac Donald (1974).

Chhabra et al. (1977) reported significant reduction in okra plants when inoculated with mixture of M. incognita and R. solani. The maximum reduction was noticed in simultaneous inoculation. Kumar and Shivkumar (1981) while studying the disease complex between Rotylenchus reniformis and R. solani on okra, reported that wilting in okra plants appeared earlier when nematode infection preceded the fungus.

Reddy et al. (1979) while studying disease complex involving M. incognita and R. solani on french bean reported that simultaneous inoculation of both the pathogens caused greater damage than either of the pathogen alone.

Khan and Husain (1988) reported that individually, R. solani was the most aggressive pathogen of cowpea followed by M. incognita and R. reniformis, while concomitance of nematode and fungus was more damaging than the association of both nematode species and the association of R. solani with M. incognita caused greater plant growth reduction as compared to its association with R. reniformis.

Junaid and Khan (1989) while working with disease complex in chickpea reported the additive effect when plants were inoculated with M. javanica and Sclerotium rolfsii. Greatest suppression was reported in simultaneous inoculation with the two pathogens.

Mehta et al. (1989) while studying interaction of M. javanica and R. solani on watermelon observed that the presence of M. javanica enhances the fungus attack while the fungus R. solani antagonistic to nematode reproduction.

De Souza and Powell (1992), while studying disease complex involving M. exigua and R. solani on coffee, observed more root necrosis and defoliation in coffee seedlings when nematode inoculation preceded the fungus as compared to when both the pathogens were inoculated either simultaneously or separately.

III, NEMATODE - FUNGUS SEEDLING DISEASE COMPLEXES

Reynolds and Hansen (1957) reported that post emergence damping off in cotton, caused by Rhizoctonia solani, became severe in the presence of M. incognita acrita. In addition to R. solani fungi such as F. oxysporum f. vasinfectum and Pythium debaryanum Hesse enhanced damping off in cotton in the presence of M. incognita acrita (Norton, 1960; White 1962; Brodie, 1963). Brodie and Cooper (1964) observed synergistic reaction between R. solani and M. incognita acrita on cotton, further, this type of reaction of R. solani with other nematodes such as Rotylenchulus reniformis, Hoplolaimus tylenchiformis Daday, 1905, M. incognita, M. hapla and M. arenaria has also been noticed. The emergence of soybean seedlings was adversely affected by R. solani in the presence of M. javanica or M. hapla (Taylor and Wyllie, 1959). Similar interaction was observed by Agarwal and Goswami (1973) when soybean seedlings were inoculated simultaneously with M. incognita and Macrophomina phaseoli (Mauble) Ashby or when nematode preceded the fungus as against those treatments where fungus preceded the nematode or where fungus alone was used. The association of Heterodera glycines and Phytophthora megasperma var. sojae caused greater damage to soybean seedlings than either of the pathogens alone (Adenji et al., 1975).

Whitney (1971, 1974) reported synergism between Heterodera schachtii and Pythium ultimum in pre and post-emergence damping-off of sugerbeet seedlings. Similar

synergism between root-knot nematode and Sclerotium rolfsii in emergence of tomato seedlings was also reported by Shukla and Swarup (1970). Raman et al. (1974) observed that seedling blight of rice caused by Sclerotium rolfsii was greater in presence of Hoploaimus indicus Sher, 1963.

Khan et al. (1971) reported much greater reduction in the emergence of cauliflower seedlings in presence of Tylenchorhynchus brassicae Siddiqui, 1961 and R. solani than in R. solani alone.

Legumes are cultivated all over the world and stand next to cereals in their economic value (Allen and Allen, 1958). Cowpea, Vigna unguiculata (Linn.) Walp. is one of the legume which is a crop of vital importance. In India, cowpea is a multipurpose crop commonly grown as vegetable, pulse, green manure and fodder. In cowpea, wilt caused by Fusarium sp. and root-rot caused by Rhizoctonia sp. are common diseases which result in great economic losses. It has also been reported that the cowpea is highly prone to damage by Meloidogyne incognita and Meloidogyne javanica (Prasad et al., 1964).

From the above review it is clear that considerable work have been carried out on disease complexes involving nematodes and fungi. During the course of survey of legume fields in Jhansi and nearby areas, severe seedling mortality

and stunting of plants was noticed in some beds of cowpea. On uprooting the plants, lesions/rotting and galls were observed on the root system. Preliminary investigations showed that the lesions were caused by Rhizoctonia solani Kuhn. and the galls by Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949. Since little information is available about the disease complex involving root-knot nematode, Meloidogyne and root-rot fungus, Rhizoctonia on cowpea, therefore, it has been considered desirable to study the following:

1. Screening of cowpea germplasms against root-knot nematode Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949.
2. Screening of the above cowpea germplasms against Rhizoctonia solani Kuhn.
3. Effect of different inoculum levels i.e. 10, 100, 1,000 and 10,000 larvae of M. incognita on plant growth of cowpea germplasm, EC-244231 (susceptible to M. incognita).
4. Effect of different inoculum levels i.e. 0.25, 0.50, 1.00 and 2.00 g of R. solani on plant growth of cowpea germplasm, EC-244231 (susceptible to R. solani).
5. Effect of inoculation of cowpea germplasm, EC-244231 (susceptible to R. solani) with -
   i) M. incognita alone;

(ii) R. solani alone;

(iii) M. incognita and R. solani simultaneously;

(iv) M. incognita 1 week prior to R. solani; and

(v) R. solani 1 week prior to M. incognita

6. Effect of inoculation of cowpea germplasm, IL-163 (resistant to R. solani) with -

(i) M. incognita alone;

(ii) R. solani alone;

(iii) M. incognita and R. solani simultaneously;

(iv) M. incognita 1 week prior to R. solani; and

(v) R. solani 1 week prior to M. incognita.

7. Effect of individual and concomitant inoculation of M. incognita and R. solani on seedling emergence/ survival of cowpea germplasm, EC-244231.

8. Effect of seed treatment with nematicide (Carbofuran) and fungicide (Bavistin) on the control of disease complex involving M. incognita and R. solani on cowpea germplasm, EC-244231.

MATERIALS AND METHODS

RAISING CULTURE AND THEIR MAINTENANCE

NEMATODE

The inoculum of root-knot nematode, Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949 was raised and maintained on brinjal seedlings (Solanum melongena L.). A single egg mass, obtained from infected roots of brinjal plant, was surface sterilized in 1:500 aqueous solution of chlorex (den Ouden, 1958) for 5 minutes and washed thrice in sterilized distilled water. The egg mass was then allowed to hatch under aseptic conditions in sterilized distilled water at 27°C ± 2°C temperature. The brinjal seedlings raised in autoclaved soil were inoculated with these larvae. In order to have regular supply of the inoculum more brinjal seedlings were inoculated at regular intervals from time to time.

Every time prior to inoculation the egg masses were removed from the infected roots of brinjal seedlings and allowed to hatch (Stemerding, 1963). For larval count, 5 ml of larval suspension thus obtained was pipetted in counting dish and the number of larvae was counted under stereoscopic microscope (Southey, 1970). The process was repeated thrice and the number of larvae present per ml of larval suspension was standardized.

Throughout the studies each seedling was inoculated with 1,000 freshly hatched larvae contained in 10 ml sterilized distilled water, unless stated otherwise.

FUNGUS

Rhizoctonia solani Kuhn used in the present studies was isolated from the infected root of cowpea (Vigna unguiculata L.) Walp. The culture was purified by making hyphal tip isolation.

The inoculum of the fungus was raised on autoclaved potato - dextrose broth (peeled and sliced potato 200 g; dextrose 20 g; distilled water 1000 ml) contained in 250 ml flasks. These flasks were incubated at 25°C ± 2°C. The mycelial mat thus obtained was filtered, washed in sterilized distilled water and then macerated in sterilized warring blender for 30 seconds (with intermitant operation of the blender). The fungal suspension was prepared by mixing the fungal mycelium in sterilized distilled water in such a way that each 10 ml of suspension contained 1 g of fungus. This suspension of the fungus was used throughout the studies, unless stated otherwise.

RAISING SEEDLINGS OF COWPEA

The seeds of cowpea germplasms used were surface sterilized with 0.1% of mercuric chloride solution and washed with sterilized distilled water thrice. The seeds were

bacterised with cowpea rhizobium culture prior to sowing.

Bacterisation of seeds was done with carrier-based culture of cowpea rhizobia, obtained from the Division of Microbiology, Indian Agricultural Research Institute, New-Delhi. The carrier-based culture was mixed with minimum amount of 10 percent sucrose solution to form a slurry and seeds were added to the slurry so as to uniformly coat the seeds with the inoculant. The seeds were dried in shade and sown immediately (Subba Rao, 1982).

INOCULATION TECHNIQUE

One week old seedlings of test plants were inoculated with root-knot nematode by pipetting equal amount of larval suspension in four holes made around the roots in the pot and with fungus by pouring mycelial suspension around the root zone.

Barring studies dealing with screening and with effect of different inoculum levels where the seedlings were inoculated with 10, 100, 1,000 and 10,000 larvae of root-knot nematode and with 0.25, 0.5, 1 and 2 g of R. solani, the test plants throughout the studies were inoculated with 1,000 larvae of root-knot and 1 g of fungus in a manner described above.

For studies dealing with concomitant inoculation following scheme was followed -

- Inoculation with nematode and fungus simultaneously;
- Inoculation with nematode 1 week prior to fungus and
- Inoculation with fungus 1 week prior to nematode.

Throughout the studies there were six replicates for each treatment. Uninoculated plants served as control.

SCREENING OF GERMPLASMS

One hundred, Exotic as well as Indian, fodder type cowpea germplasms obtained from Indian Grassland and Fodder Research Institute, JHANSI were screened against *M. incognita* and *R. solani*.

AGAINST NEMATODE

For screening against *M. incognita*, surface sterilized seeds of the germplasms were sown in plastic glasses containing autoclaved river-bed sand. On emergence five days old seedlings were inoculated with 200 freshly hatched juveniles of root-knot nematode by pipetting the larval suspension around the root-zone. Seedlings were nursed with nutrient solution (Long-Ashton) once a day. After 14 days of inoculation the seedlings were carefully uprooted and thoroughly washed in running water. The root system of each seedling was cut and stained in 0.1% boiling lectophenol acid fuchsin and then cleared in lectophenol for

24 hours (Mc Beth et al., 1941). Stained roots were examined for penetration and developmental stages of the nematode. The number of larvae in different stages inside the roots were counted by teasing the roots under stereoscopic microscope. On the basis of percent penetration and development of the nematode in different stages inside the roots, the cowpea germplasms tested were rated according to the following scale -

Rating	Reaction type	% penetration of larvae	% of larvae developed in III stage, IV stage & young female
1	Highly resistant (HR)	1 - 25	1 - 25
2	Resistant (R)	26 - 50	26 - 50
3	Susceptible (S)	51 - 75	51 - 75
4	Highly susceptible (HS)	76 - 100	76 - 100

AGAINST FUNGUS

The screening against R. solani was done by blotter paper technique. The inoculum of the fungus was raised as described earlier. The fungus suspension obtained from a five days old culture of a flask was used to inoculate 10 germplasms of cowpea at a time. The seedlings for inoculation were raised from surface sterilized seeds in 15 cm pots containing autoclaved river-bed sand. Seedlings were nursed with nutrient solution for five days. Five days old seed-

lings were uprooted, root system was washed in running water and rinsed in sterilized distilled water. The root system of 15 seedlings of a germplasm were dipped in the inoculum of the fungus contained in a beaker with an up and down movement for about 30 seconds. The excess of inoculum was removed by touching the edge of the beaker. The seedlings of the germplasm thus inoculated were placed side by side on a white blotter paper (size 45 cm x 25 cm with one fold) so that only the cotyledons and roots are covered and the green tops of the seedlings remain outside the blotter paper after it is folded. Five uninoculated seedlings were also kept as check. The folded blotter paper was moistened adequately with sterilized water. The folded blotter papers were kept one on top of the other, in heeps of ten in a tray. The trays so prepared were kept in B.O.D. at $35^{o}C \pm 2^{o}C$ for a week. 12 hours artificial light have been provided and the blotters were moistened adequately daily. After a week of incubation, the seedlings were examined for the extent of root damage caused by the fungus. The seedlings were scored according to the following rating scale used at ICRISAT (Nene et al., 1981).

Rating	Root-rot	Reaction type
1	No infection on roots	Resistant (R)
2	Very few small lesions on roots	Moderately resistant (MR)
3	Lesions on roots clear but small, new roots free from infection	Tolerant (T)

4	Lesions on roots many; new roots generally free from lesions.	Moderately susceptible (MS)
5	Roots infected and completely discoloured.	Susceptible (S)

RECORDING OF DATA

Plants were carefully removed from the pots 45 days after inoculation. Roots were thoroughly washed in slow running water to remove the adhering soil particles. The excess of water was removed by using blotting paper. Utmost care was taken not to disturb the root system during the entire operation.

PLANT GROWTH

The growth of the plant was determined by recording length, fresh and dry weight of the root and shoot. The reduction in plant growth was also calculated in percent.

ROOT-KNOT INDEX

Root-knot index was recorded according to the following scale proposed by Khan (1971).

0 = No infection

1 = Trace to light infection

2 = Light to moderate infection

3 = Moderate to heavy infection

4 = Very severe infection

ROOT-NODULE INDEX

The nodule index was recorded according to the following scale.

0 = Nil

1 = Few

2 = Light

3 = Moderate

4 = High

NEMATODE POPULATION

At the end of each experiment nematode population was estimated by counting different stages of larvae in the root and larvae present in the soil.

In root

For counting the different stages of nematode larvae in the root, infected roots were carefully washed and then 1 g homogeneous mass of the root taken out per root system, was macerated in warring blender for 30 seconds and the different stages of the nematode were counted in the suspension under stereoscopic microscope.

In soil

For counting the nematode population in soil, the soil of the pot was mixed thoroughly and a sub-sample of 200 g of soil per pot was put in a large bucket containing

water and stirred until all clods were broken. The heavy soil particles sank to the bottom and the nematode larvae remained suspended in water. The water was then poured into another bucket through a coarse sieve (60 mesh), leaving the heavy particles in the bucket. The whole aliquot was then passed through 350 mesh sieve. The catch of the sieve was taken in a beaker and the quantity of water was reduced by decantation allowing sufficient time for the nematode larvae to settle down. The suspension containing the nematode larvae was poured over tissue paper appended to a coarse sieve contained in a trough in which the sieve just touched the water. After 24 hours most of the nematode larvae wriggled out at the bottom of the trough in clear water. These were later concentrated by decantation. The nematode larvae were counted with the help of counting dish under stereoscopic microscope.

EFFECT OF CONCOMITANT INOCULATION ON SEEDLINGS EMERGENCE

For studying the effect of concomitant inoculation on the seedling emergence, autoclaved soil was inoculated with 1,000 and 5,000 larvae of root-knot nematode and with 1 g and 5 g of fungus/kg of soil separately and with nematode and fungus together in different combinations. Uninoculated soil served as control. In each pot, 10 surface sterilized seeds of cowpea germplasm EC-244231 (susceptible to both the

pathogens) were sown. After one week of sowing seedling emergence and after two weeks survival of seedlings was recorded. For each treatment there were three replicates.

EFFECT OF NEMATICIDE/FUNGICIDE ON THE CONTROL OF DISEASE COMPLEX

For studies dealing with the effect of nematicide/fungicide on the control of disease complex, surface sterilized and bacterised seeds of the cowpea germplasm EC-244231 were treated with nematicide (Carbofuran)/fungicide (Bavistin) prior to sowing as under.

Carbofuran treatment

Surface sterilized and bacterised seeds were treated with Carbofuran 3G @ 2% a.i. w/w using gum arebic as adhesive.

Bavistin treatment

Surface sterilized and bacterised seeds were treated with 0.2 g Bavistin powder using gum arebic as adhesive.

First of all gum was added to weighed quantity of seeds, mixed well to make uniform coating over the seeds. The required quantity of nematicide/fungicide sprinkled over it to give uniform coating over the seeds with the sticker. These treated seeds were spread in the shade and dried.

One week old seedlings raised from both treated and untreated seeds were inoculated individually and concomitantly with M. incognita (1,000 larvae/plant) and R. solani (1 g fungus/plant). Untreated uninoculated plants served as control. There were six replicates of each treatment.

The data obtained were analysed statistically.

EXPERIMENTAL RESULTS

SCREENING OF COWPEA GERMPLASMS

AGAINST M. INCOGNITA

(Table 1)

The data as given in table (1) show that when one hundred Exotic as well as Indian fodder type cowpea germplasms, viz., EC-244426, EC-244250, EC-244427, EC-244270, EC-244241, EC-244256, EC-244430, EC-244317, EC-242416, EC-244209, EC-244415, EC-244249, EC-244310, EC-244438, EC-244211, EC-244131, EC-244424, EC-244320, EC-244243, EC-244123, EC-48543, EC-244432, EC-244226, EC-244231, EC-244125, EC-4166, EC-8414, EC-37614, EC-244372, EC-244242, IC-6805, IC-48720, IC-19070, IC-39354, IC-28671, IC-42194, IC-68661, IC-20737-A, IC-20683/83, IC-20458/PI, IC-20677, IC-20504, IC-20504-2, IC-20533, IL-133, NP-3, IC-19785, EC-244409, IL-1108, IL-1057, IL-362, IL-178-B, Hy 8 P68-A, Hy 10 P68-1, IL-1035, Hy 10 P68-2, IL-380, NP 3-18, NP 3-17, IL-390-B, NP 3-16, IL-672-A, IL-163, Hy 10 P68-4, Hy 8 (60-15), NP 3-2, IL-8705, NP 3-1, IL-1146-A, IL-904-B, Hy 78 P5/A, IL-855, IL-1149, Hy 6 P52-15, IL-1116, Hy 78 P5, IL-672, IL-1066, NP 3-9, IL-3156, Hy 10 P36-1, IL-876, IL-3157, IL-499-A, IL-200, IL-449, NP 20, IL-1093, Hy 8 P60-9, IL-178-A, IL-3186, IL-893, IL-914, Hy 1 P65, Hy 10 P68, IL-450, IL-1155-A, IL-1059-A, Hy 8 P60-12 and Hy 8 P6-13 were inoculated with 200 freshly hatched juveniles of M. incognita, the different germplasms exhibited different degree of resistance and susceptibility as measured on the basis of percent penetration

Table 1: Screening of fodder type cowpea germplasms against *M. incognita* (Kofoid & White, 1919) Chitwood, 1949 (Based on percent penetration and development of nematode)

Sl. No.	Cowpea germplasm	% larval penetration	Rating scale	Reaction type
1	2	3	4	5
1.	EC - 244426	56.5(67.25)	3	S
2.	EC - 244250	43.0(25.58)	2	R
3.	EC - 244427	41.5(30.12)	2	R
4.	EC - 244270	71.0(64.78)	3	S
5.	EC - 244241	50.0(35.00)	2	R
6.	EC - 244256	47.0(31.91)	2	R
7.	EC - 244430	31.5(31.74)	2	R
8.	EC - 244317	27.0(27.77)	2	R
9.	EC - 242416	35.0(28.57)	2	R
10.	EC - 244209	25.0(18.00)	1	HR
11.	EC - 244415	83.0(78.31)	4	HS
12.	EC - 244249	32.0(28.12)	2	R
13.	EC - 244310	71.5(69.93)	3	S
14.	EC - 244438	29.5(30.50)	2	R
15.	EC - 244211	73.0(65.06)	3	S
16.	EC - 244131	17.5(8.57)	1	HR
17.	EC - 244424	42.0(29.76)	2	R
18.	EC - 244320	29.5(33.89)	2	R
19.	EC - 244243	76.0(78.94)	4	HS
20	EC - 244123	12.5(0.0)	1	HR

contd.

1	2	3	4	5
21.	EC - 48543	94.0(84.04)	4	HS
22.	EC - 244432	78.0(76.92)	4	HS
23.	EC - 244226	68.0(66.17)	3	S
24.	EC - 244231	55.0(63.63)	3	S
25.	EC - 244125	47.0(31.91)	2	R
26.	EC - 4166	80.0(84.37)	4	HS
27.	EC - 8414	43.0(34.88)	2	R
28.	EC - 37614	25.0(16.00)	1	HR
29.	EC - 244372	28.0(25.78)	2	R
30.	EC - 244242	55.0(59.09)	3	S
31.	IC - 6805	42.0(35.71)	2	R
32.	IC - 48720	30.0(26.66)	2	R
33.	IC - 19070	29.5(32.20)	2	R
34.	IC - 39354	90.0(83.33)	4	HS
35.	IC - 28671	38.0(32.89)	2	R
36.	IC - 42194	41.0(32.92)	2	R
37.	IC - 68661	30.0(33.33)	2	R
38.	IC - 20737-A	49.0(33.67)	2	R
39.	IC - 20683/83	41.5(36.14)	2	R
40.	IC - 20458/PI	66.0(68.18)	3	S
41.	IC - 20677	43.0(29.06)	2	R
42.	IC - 20504	42.5(35.29)	2	R
43.	IC - 20504-2	20.0(17.50)	1	HR

contd.

1	2	3	4	5
44.	IC - 20533	39.0(35.89)	2	R
45.	IL - 133	48.5(36.08)	2	R
46.	NP - 3	44.0(36.36)	2	R
47.	IC - 19785	28.0(26.78)	2	R
48.	EC - 244409	23.0(17.39)	1	HR
49.	IL - 1108	48.0(30.20)	2	R
50.	IL - 1057	38.0(32.89)	2	R
51.	IL - 362	52.5(52.38)	3	S
52.	IL - 178 - B	49.5(30.30)	2	R
53.	Hy 8 P68 - A	34.5(28.98)	2	R
54.	Hy 10 P68 - 1	61.5(65.04)	3	S
55.	IL - 1035	73.5(69.38)	3	S
56.	Hy 10 P68 - 2	30.5(27.86)	2	R
57.	IL - 380	68.0(66.17)	3	S
58.	NP 3 - 18	23.5(19.14)	1	HR
59.	NP 3 - 17	23.5(21.27)	1	HR
60.	IL - 390 - B	49.0(31.71)	2	R
61.	NP 3 - 16	20.5(14.63)	1	HR
62.	IL - 672 - A	29.0(31.03)	2	R
63.	IL - 163	58.5(64.10)	3	S
64.	Hy 10 P68 - 4	30.0(31.66)	2	R
65.	Hy 8 P60 - 15	17.0(11.76)	1	HR
66.	NP 3 - 2	41.5(30.12)	2	R

contd.

1	2	3	4	5
67.	IL - 8705	27.0(25.92)	2	R
68.	NP 3 - 1	19.5(15.38)	1	HR
69.	IL - 1146 - A	8.5(0.0)	1	HR
70.	IL - 904 - B	72.5(65.51)	3	S
71.	Hy 78 P5/A	27.0(27.77)	2	R
72.	IL - 855	32.5(30.76)	2	R
73.	IL - 1149	63.5(70.86)	3	S
74.	Hy 6 P52 - 15	44.5(33.70)	2	R
75.	IL - 1116	40.5(30.86)	2	R
76.	Hy 78 P5	44.0(28.40)	2	R
77.	IL - 672	40.5(32.09)	2	R
78.	IL - 1066	58.5(61.52)	3	S
79.	NP 3 - 9	17.5(8.57)	1	HR
80.	IL - 3156	39.0(32.05)	2	R
81.	Hy 10 P36-1	47.0(35.10)	2	R
82.	IL - 876	18.0(11.11)	1	HR
83.	IL - 3157	43.0(29.06)	2	R
84.	IL - 499 - A	34.0(27.94)	2	R
85.	IL - 200	17.5(8.57)	1	HR
86.	IL - 449	43.0(25.58)	2	R
87.	NP 20	44.0(32.95)	2	R
88.	IL - 1093	22.0(13.63)	1	HR
89.	Hy 8 P60 - 9	43.0(32.55)	2	R

contd.

1	2	3	4	5
90.	IL - 178 - A	58.5(68.37)	3	S
91.	IL - 3186	36.0(30.55)	2	R
92.	IL - 893	26.5(28.30)	2	R
93.	IL - 914	49.0(30.61)	2	R
94.	Hy 1 P65	19.5(12.82)	1	HR
95.	Hy 10 P68	19.5(10.25)	1	HR
96.	IL - 450	18.5(13.51)	1	HR
97.	IL - 1155-A	17.0(5.88)	1	HR
98.	IL - 1059-A	28.5(26.55)	2	R
99.	Hy 8 P60-12	49.5(30.30)	2	R
100.	Hy 8 P6-13	29.0(25.86)	2	R

EC = Exotic Collection; IC = Indian Collection; NP = New Puda
IL = Indigenous Legume (IGFRI); Hy P = Hybrid Plant;
HR = Highly Resistant; R = Resistant; S = Susceptible;
HS = Highly Susceptible.

N.B.- 1. Each reading is a mean of three replicates.

2. Figures in parentheses denotes % larval development in III stage, IV stage and young female.

and development of the nematode in the roots, after 14 days of inoculation. The results indicate that out of 100 germplasms tested 20 were proved to be highly resistant (Sl.No. 10, 16, 20, 28, 43, 48, 58, 59, 61, 65, 68, 69, 79, 82, 85, 88, 94, 95, 96 and 97); 57 to be resistant (Sl.No. 2, 3, 5, 6, 7, 8, 9, 12, 14, 17, 18, 25, 27, 29, 31, 32, 33, 35, 36, 37, 38, 39, 41, 42, 44, 45, 46, 47, 49, 50, 52, 53, 56, 60, 62, 64, 66, 67, 71, 72, 74, 75, 76, 77, 80, 81, 83, 84, 86, 87, 89, 91, 92, 93, 98, 99 and 100); 17 to be susceptible (Sl.No. 1, 4, 13, 15, 23, 24, 30, 40, 51, 54, 55, 57, 63, 70, 73, 78 and 90) and 6 to be highly susceptible (Sl.No. 11, 19, 21, 22, 26 and 34).

AGAINST R. SOLANI

(Table 2)

The data as given in table (2) show that when all the above Exotic as well as Indian cowpea germplasms were screened against R. solani by blotter paper technique, the different germplasms show different reactions as measured on the basis of extent of damage caused by the fungus in the root system. The results show that out of the hundred germplasms tested, 14 were found to be resistant (Sl.No. 6,

Table 2: Screening of fodder type cowpea germplasms against *R. solani* Kuhn.
(Based on extent of damage caused on the root system)

Sl.No.	Cowpea germplasm	Rating scale	Reaction type
1	2	3	4
1.	EC - 244426	3	T
2.	EC - 244250	4	MS
3.	EC - 244427	2	MR
4.	EC - 244270	2	MR
5.	EC - 244241	2	MR
6.	EC - 244256	1	R
7.	EC - 244430	2	MR
8.	EC - 244317	1	R
9.	EC - 242416	1	R
10.	EC - 244209	2	MR
11.	EC - 244415	1	R
12.	EC - 244249	1	R
13.	EC - 244310	2	MR
14.	EC - 244438	3	T
15.	EC - 244211	3	T
16.	EC - 244131	2	MR
17.	EC - 244424	3	T
18.	EC - 244320	3	T
19.	EC - 244243	2	MR
20.	EC - 244128	2	MR

Contd.

1	2	3	4
21.	EC - 48543	2	MR
22.	EC - 244432	3	T
23.	EC - 244226	1	R
24.	EC - 244231	5	S
25.	EC - 244125	5	S
26.	EC - 4166	4	MS
27.	EC - 8414	3	T
28.	EC - 37614	3	T
29.	EC - 244372	3	T
30.	EC - 244242	2	MR
31.	IC - 6805	2	MR
32.	IC - 48720	3	T
33.	IC - 19070	3	T
34.	IC - 39354	4	MS
35.	IC - 28671	4	MS
36.	IC - 42194	3	T
37.	IC - 68661	2	MR
38.	IC - 20737-A	3	T
39.	IC - 20683/83	4	MS
40.	IC - 20458/PI	3	T
41.	IC - 20677	4	MS
42.	IC - 20504	4	MS
43.	IC - 20504-2	3	T

Contd.

1	2	3	4
44.	IC - 20533	5	S
45.	IL - 133	2	MR
46.	NP - 3	3	T
47.	IC - 19785	3	T
48.	EC - 244409	4	MS
49.	IL - 1108	5	S
50.	IL - 1057	5	S
51.	IL - 362	3	T
52.	IL - 178 - B	3	T
53.	Hy 8 P68 - A	3	T
54.	Hy 10 P68 - 1	3	T
55.	IL - 1035	3	T
56.	Hy 10 P68 - 2	2	MR
57.	IL - 380	1	R
58.	NP 3 - 18	1	R
59.	NP 3 - 17	2	MR
60.	IL - 390 - B	3	T
61.	NP 3 - 16	3	T
62.	IL - 672 - A	1	R
63.	IL - 163	1	R
64.	Hy 10 P68 - 4	2	MR
65.	Hy 8 P60 - 15	2	MR
66.	NP 3 - 2	1	R
67.	IL - 8705	1	R

Contd.

1	2	3	4
68.	NP 3 - 1	1	R
69.	IL - 1146 - A	2	MR
70.	IL - 904 - B	2	MR
71.	Hy 78 P5/A	3	T
72.	IL - 855	2	MR
73.	IL - 1149	2	MR
74.	Hy 6 P52 - 15	3	T
75.	IL - 1116	3	T
76.	Hy 78 P5	3	T
77.	IL - 672	1	R
78.	IL - 1066	2	MR
79.	NP 3 - 9	3	T
80.	IL - 3156	3	T
81.	Hy 10 P36 - 1	2	MR
82.	IL - 876	3	T
83.	IL - 3157	4	MS
84.	IL - 499-A	3	T
85.	IL - 200	2	MR
86.	IL - 449	3	T
87.	NP 20	3	T
88.	IL - 1093	3	T
89.	Hy 8 P60-9	3	T
90.	IL - 178-A	3	T

contd.

1	2	3	4
91.	IL - 3186	2	MR
92.	IL - 893	2	MR
93.	IL - 914	4	MS
94.	Hy 1 P65	3	T
95.	Hy 10 P68	4	MS
96.	IL - 450	3	T
97.	IL - 1155-A	3	T
98.	IL - 1059-A	3	T
99.	Hy 8 P60-12	2	MR
100.	Hy 8 P6-13	2	MR

EC = Exotic Collection; IC = Indian Collection; NP = New Pusa;
IL = Indigenous Legume (IGFRI); Hy P = Hybrid Plant;
R = Resistant; MR = Moderately resistant; T = Tolerant;
MS = Moderately susceptible; S = Susceptible

8, 9, 11, 12, 23, 57, 58, 62, 63, 66, 67, 68 and 77); 29 to be moderately resistant (Sl.No. 3, 4, 5, 7, 10, 13, 16, 19, 20, 21, 30, 31, 37, 45, 56, 59, 64, 65, 69, 70, 72, 73, 78, 81, 85, 91, 92, 99 and 100); 41 to be tolerant (Sl.No. 1, 14, 15, 17, 18, 22, 27, 28, 29, 32, 33, 36, 38, 40, 43, 46, 47, 51, 52, 53, 54, 55, 60, 61, 71. 74, 75, 76, 79, 80, 82, 84, 86, 87, 88, 89, 90, 94, 96, 97 and 98); 11 moderately susceptible (Sl.No. 2, 26, 34, 35, 39, 41, 42, 48, 83, 93 and 95) and 5 to be susceptible (Sl.No. 24, 25, 44, 49 and 50).

Out of the above, two germplasms namely, EC-244231 (susceptible to R. solani) and IL-163 (resistant to R. solani) have been selected for subsequent studies, keeping in view that these both are susceptible to M. incognita.

EFFECT OF DIFFERENT INOCULUM LEVELS OF M. INCOGNITA

(Table 3; Figs. 1&2; Appendix I)

The data given in table (3) show that when seedlings of cowpea germplasm, EC-244231 (susceptible to R. solani) were inoculated with 10, 100, 1,000 and 10,000 larvae of M. incognita, the dry weight of the plants, 45 days after inoculation, was 6.02, 5.70, 4.80 and 3.40 g respectively as against 6.86 g of uninoculated plants. The final nematode population for the corresponding inoculum levels was 260, 809, 1758 and 5421 respectively. The root-knot index was 1.00, 1.30, 2.00 and 3.30 respectively.

Table 3: Effect of four different inoculum levels of M. incognita on plant growth, nodulation, root-knot index and nematode population, 45 days after inoculation of seedlings of cowpea germplasm, EC-244231 (susceptible to M. incognita)

Treatment	Plant dry weight (g)		Nodule index	% reduction over uninoculated	Root-knot index	Nematode population		
	Root + shoot	% reduction over uninoculated				Per g of root	Per 200 g of soil	Total population
Uninoculated	6.86	-	3.80	-	-	-	-	-
10 larvae	6.02	12.24	3.16	16.84	1.00	75	185	260
100 larvae	5.70	16.90	2.60*	31.57	1.30	276	533	809
1,000 larvae	4.80*	30.02	2.30*	39.47	2.00	370	1388	1758
10,000 larvae	3.40*	50.43	1.90*	50.00	3.30	2450	2971	5421
C.D. at 5%	1.05	-	0.59	-	0.72	-	-	-
C.D. at 1%	1.48	-	0.83	-	1.01	-	-	-

Each reading is an average of 3 replicates.
*Significant at 1% level against uninoculated.
N.B. - For details please see Appendix I.

Fig. 1: Plant dry weight, nodule index, root-knot index and nematode population when seedlings of cowpea germplasm, EC-244231 were inoculated with four different inoculum levels of *M. incognita*.

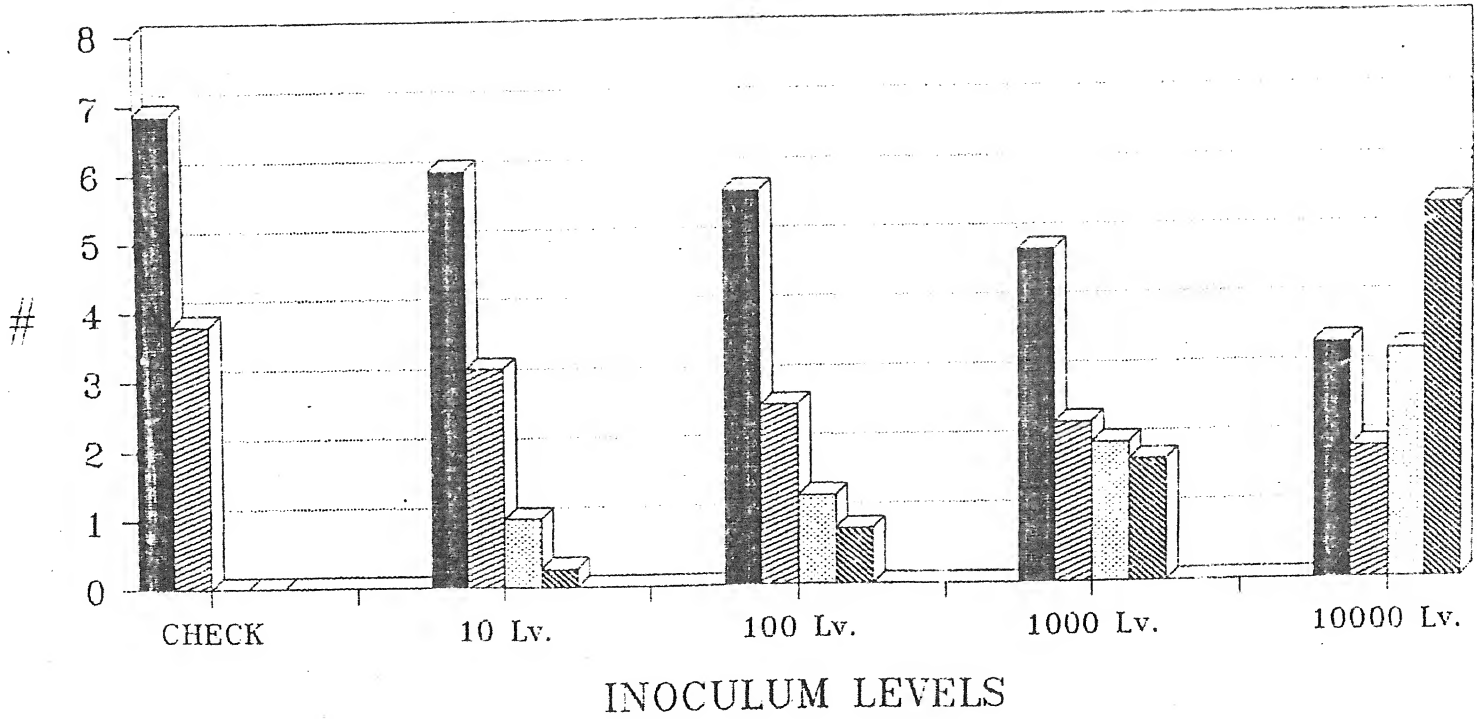

8
7
6
5
4
3
2
1
0
#
CHECK
10 Lv.
100 Lv.
1000 Lv.
10000 Lv.
INOCULUM LEVELS
DRY WEIGHT (g)
ROOT-KNOT INDEX
NODULE INDEX
NEMATODE POPULATION
DRY WEIGHT(g)/NODULE INDEX/ROOT-KNOT INDEX/NEMATODE POPULATION (X1000)

FIG. 1

Fig. 2: Root growth, nodulation and root-knot index when seedlings were inoculated with four different inoculum levels of *M. incognita*. CHECK = Uninoculated.

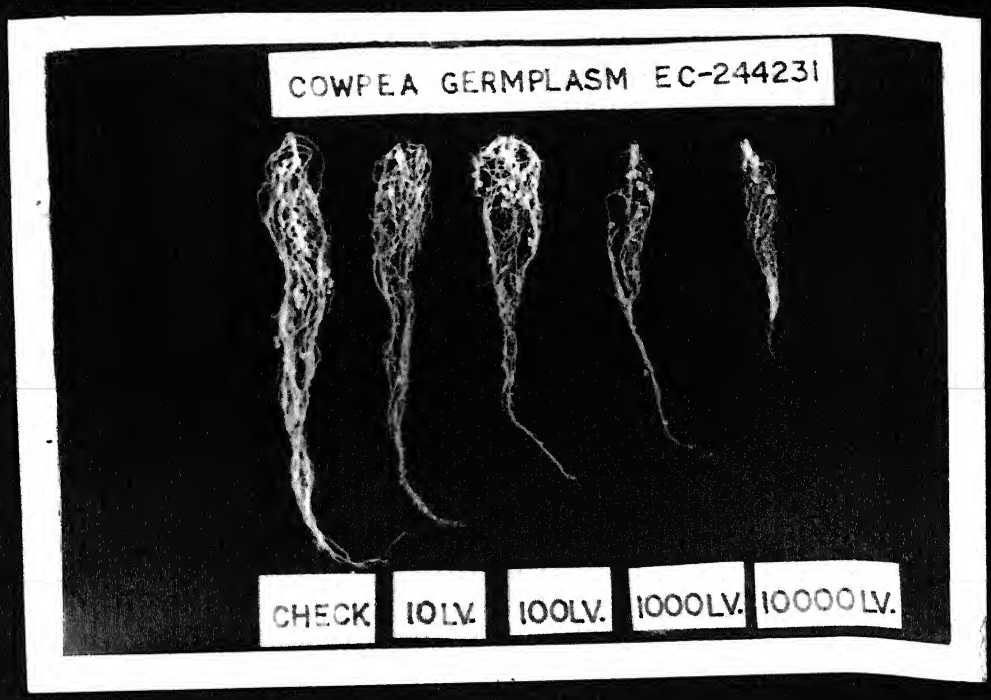

COWPEA GERMPLASM EC-244231
CHECK
10 LV.
100 LV.
1000 LV.
10000 LV.

FIG. 2

It is, therefore, clear that with an increase in inoculum level there was a corresponding decrease in dry weight and increase in the nematode population and root-knot index. On the other hand, the nodule index was reduced with an increase in inoculum level and it was 3.16, 2.60, 2.30 and 1.90 respectively for the corresponding inoculum levels as against 3.80 in uninoculated plants.

1,000 larvae were sufficient to cause significant reduction (at 1% level) in dry weight of the plants against uninoculated one.

EFFECT OF DIFFERENT INOCULUM LEVELS OF R. SOLANI
(Table 4; Figs. 3&4; Appendix II)

The data given in table (4) show that when the seedlings of cowpea germplasm, EC-244231 were inoculated with 0.25, 0.50, 1.00 and 2.00 g of R. solani, the dry weight of the plants, after 45 days of inoculation, was 6.20, 5.86, 4.89 and 4.00 g respectively as against 6.86 of uninoculated plants. The nodule index was 3.50, 3,30, 2.80 and 2.50 respectively as against 3.80 in uninoculated plants for the corresponding levels.

It is evident from the data that with an increase in inoculum level there was a corresponding decrease in dry weight and nodule index.

Table 4: Effect of four different inoculum levels of R. solani on plant growth and nodulation, 45 days after inoculation of seedlings of cowpea germplasm, EC-244231 (Susceptible to R. solani)

Treatment	Plant dry weight (g)		Nodule index	% reduction over uninoculated
	Root + shoot	% reduction over uninoculated		
Uninoculated	6.86	-	3.80	-
0.25 g	6.20	9.62	3.50	7.89
0.50 g	5.86	14.57	3.30	13.15
1.00 g	4.89*	28.71	2.80*	26.31
2.00 g	4.00*	41.69	2.50*	34.21
C.D. at 5%	0.76	-	0.49	-
C.D. at 1%	1.06	-	0.68	-

Each reading is an average of 3 replicates.

* Significant at 1% level against uninoculated.

N.B. - For details please see Appendix II.

Fig. 3: Plant dry weight and nodule index when seedlings of cowpea germplasm, EC-244231 were inoculated with four different inoculum levels of *R. solani*.

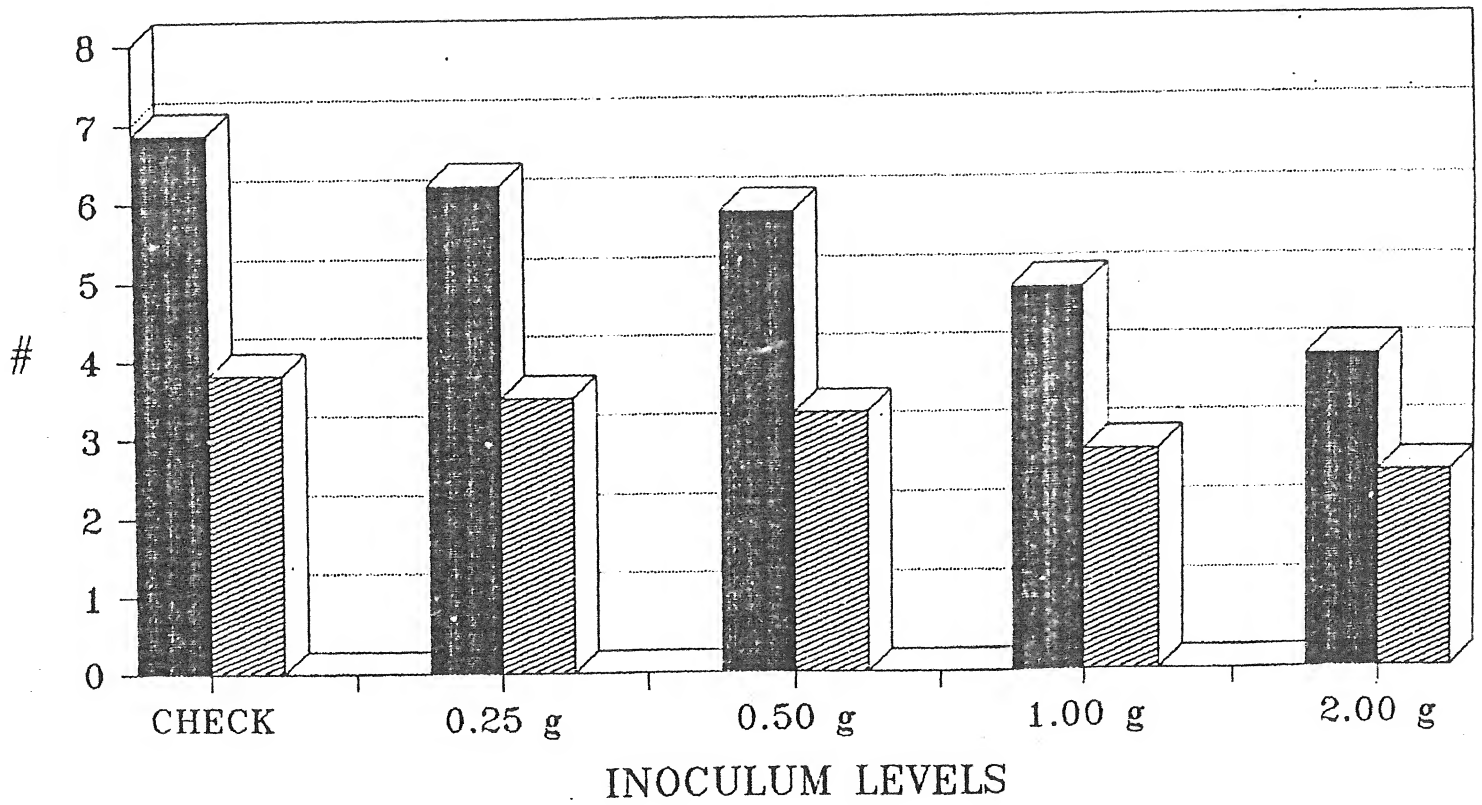

8
7
6
5
4
3
2
1
0
#
CHECK
0.25 g
0.50 g
1.00 g
2.00 g
INOCULUM LEVELS
DRY WEIGHT (g)
NODULE INDEX
DRY WEIGHT(g)/NODULE INDEX

FIG. 3

Fig. 4: Root growth and nodulation when seedlings were inoculated with four different inoculum levels of R. solani
CHECK = Uninoculated.

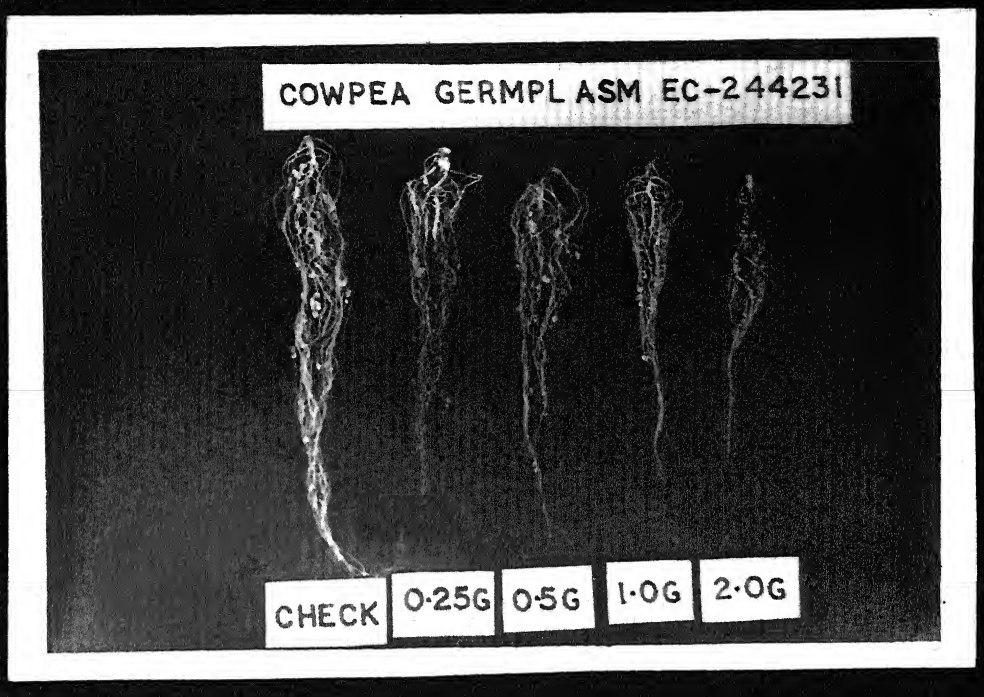


FIG. 4

Table 5: Resu[illegible] solani) with 1,000 larva[illegible]ent combinations) on plant[illegible]oculation

Treatment	[illegible]atode population		R = Pf/Pi
	[illegible] 200 g [illegible] soil	Total popu-lation	
Uninoculated	[illegible] -	-	-
Nematode alone	[illegible]1793	3425	3.425
Fungus alone	[illegible] -	-	-
Nematode & fungus simultaneously	[illegible]1303	2349	2.349*
Nematode 1 week prior to fungus	[illegible]1846	2683	2.683*
Fungus 1 week prior to nematode	[illegible]1190	1988	1.988*
C.D. at 5% level	[illegible] -	-	0.025
C.D. at 1% level	[illegible] -	-	0.036

Each readin

*Significant

●Significant

N.B. - For d

The 1 g of fungus R. solani was sufficient to cause significant reduction (at 1% level) in dry weight of the plants against uninoculated one.

INOCULATION WITH M. INCOGNITA AND R. SOLANI

Cowpea germplasm EC-244231 (susceptible to R. solani)
(Table 5; Figs. 5-8; Appendix III)

The results given in table (5) show that when seedlings of cowpea germplasm, EC-244231 were inoculated with root-knot nematode alone or with fungus alone or with mixture of the two simultaneously or sequentially with nematode 1 week prior to fungus or fungus 1 week prior to nematode, the percent reduction in dry weight, 45 days after inoculation, over uninoculated one in the above treatments was 27.34, 29.46, 58.93, 43.29 and 32.65 g respectively; root-knot index was 2.90, 0, 2.60, 2.80 and 2,50 respectively and the final nematode population was 3425, 0, 2349, 2683, and 1988 respectively for the corresponding treatments.

Marked reduction in nodule index was also observed for the corresponding treatments and it was 2.40, 2.66, 2.10, 2.26 and 2.40 as against 3.70 in uninoculated plants.

From the results presented above, it is clear that in all the treatments reduction in dry weight was significant (at 1% level) as against uninoculated. When C.D. at 1% level was calculated in between the concomitant treatments, it was

Table 5: Results of inoculating seedlings of cowpea germplasm, EC-244231 (susceptible to R. solani) with 1,000 larvae of M. incognita and 1 g of R. solani singly and concomitantly (three different combinations) on plant growth, nodulation, root-knot index and nematode population, 45 days after inoculation

Treatment	Plant dry weight (g)		Nodule index	% reduction over uninoculated	Root-knot index	% reduction over nematode alone	Nematode population			R = Pf/Pi
	Root + shoot	% reduction over uninoculated					Per g of root	per 200 g of soil	Total population	
Uninoculated	9.40	-	3.70	-	-	-	-	-	-	-
Nematode alone	6.83*	27.34	2.40*	35.13	2.90	-	1632	1793	3425	3.425
Fungus alone	6.63*	29.46	2.66*	28.10	-	-	-	-	-	-
Nematode & fungus simultaneously	3.86*●	58.93	2.10*	43.22	2.60*	10.34	1046	1303	2349	2.349*
Nematode 1 week prior to fungus	5.33*	43.29	2.26*	38.91	2.80	3.44	837	1846	2683	2.683*
Fungus 1 week prior to nematode	6.33*	32.65	2.40*	35.13	2.50*	13.79	798	1190	1988	1.988*
C.D. at 5% level	0.88	-	0.31	-	0.14	-	-	-	-	0.025
C.D. at 1% level	1.23	-	0.43	-	0.20	-	-	-	-	0.036

Each reading is an average of 3 replicates

*Significant at 1% level against uninoculated

●Significant at 1% level in between the concomitant treatments

N.B.- For details please see Appendix III.

Fig. 5: Plant dry weight, nodule index, root-knot index and nematode population when seedlings of cowpea germplasm IC-244231 were inoculated with 1,000 larvae of M. incognita and 1 g of F. solani individually and concomitantly (three different combinations)

A = Uninoculated; B = Nematode alone; C = Fungus alone; D = Nematode and fungus simultaneously; E = Nematode 1 week prior to fungus; F = Fungus 1 week prior to nematode.

Fig. 5: Plant dry weight, nodule index, root-knot index and nematode population when seedlings of cowpea germplasm IC-244231 were inoculated with 1,000 larvae of *M. incognita* and 1 g of *F. solani* individually and concomitantly (three different combinations)

A = Uninoculated; B = Nematode alone; C = Fungus alone; D = Nematode and fungus simultaneously; E = Nematode 1 week prior to fungus; F = Fungus 1 week prior to nematode.

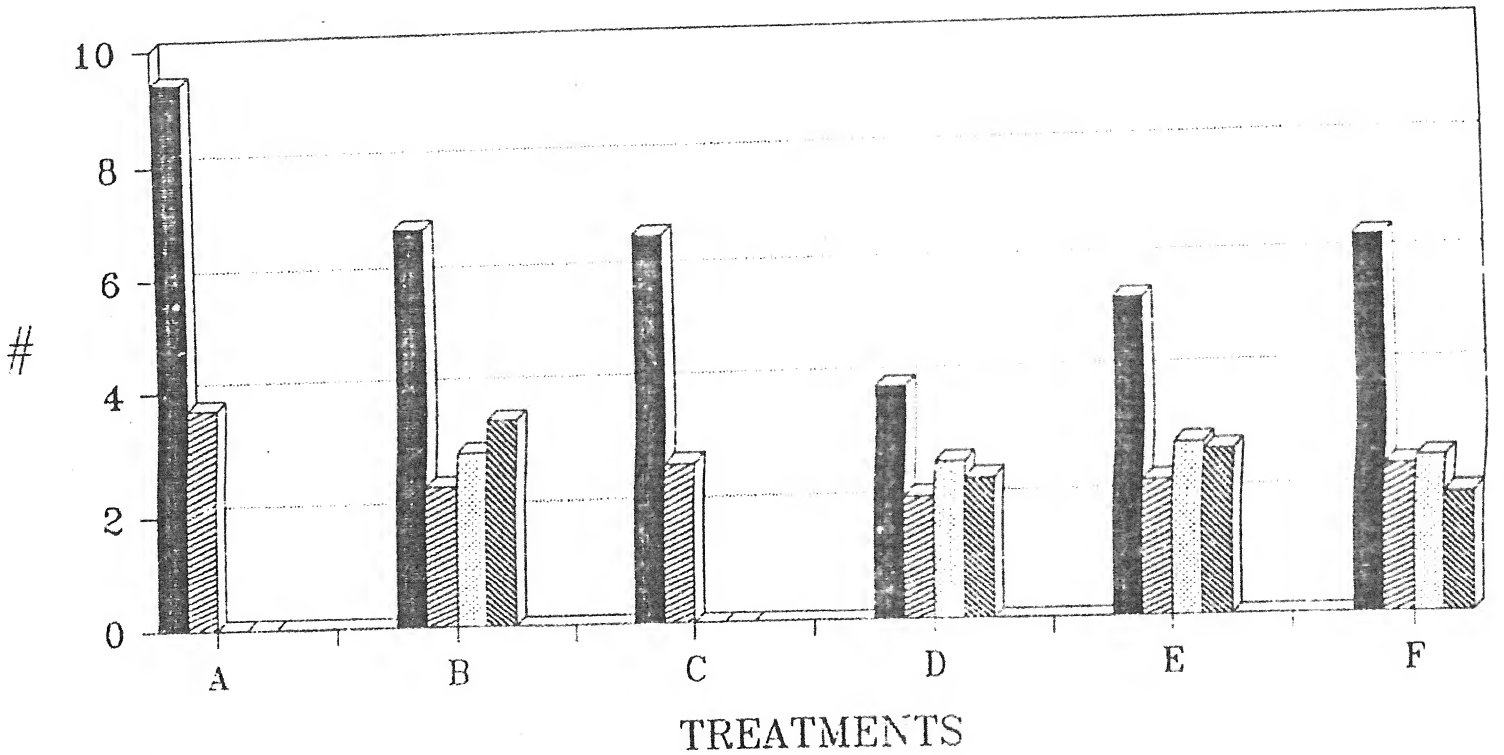

10
8
6
4
2
0
#
A
B
C
D
E
F
TREATMENTS
DRY WEIGHT (g)
ROOT-KNOT INDEX
NODULE INDEX
NEMATODE POPULATION
DRY WEIGHT(g)/NODULE INDEX/ROOT-KNOT INDEX/NEMATODE POPULATION (X1000)

FIG. 5

Fig. 6: Plant growth, nodulation and root-knot index when seedlings were inoculated with 1,000 larvae of *M. incognita* and 1 g of *R. solani* simultaneously

CHECK = Uninoculated

N = Nematode alone

F = Fungus alone

N + F = Nematode and fungus simultaneously

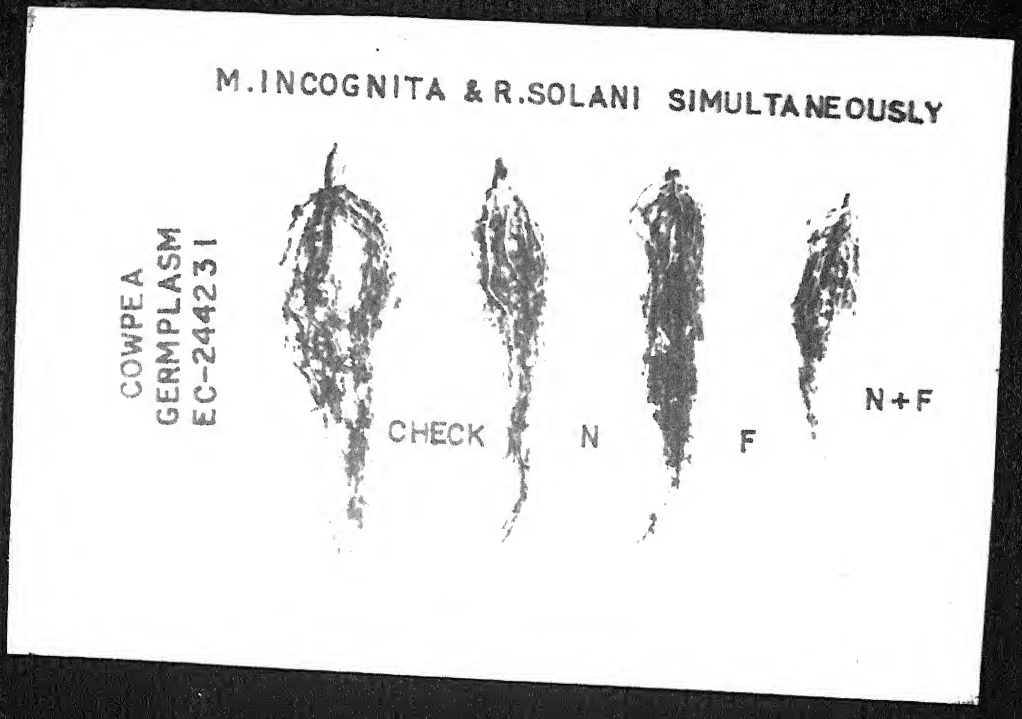

M. INCOGNITA & R. SOLANI SIMULTANEOUSLY
COWPEA
GERMPLASM
EC-244231
CHECK
N
F
N+F

FIG. 6

Fig. 7: Root growth, nodulation and root-knot index when seedlings were inoculated with 1,000 larvae of *M. incognita* 1 week prior to 1 g of *R. solani*
CHECK = Uninoculated
N = Nematode alone
F = Fungus alone
N → F = Nematode prior to fungus

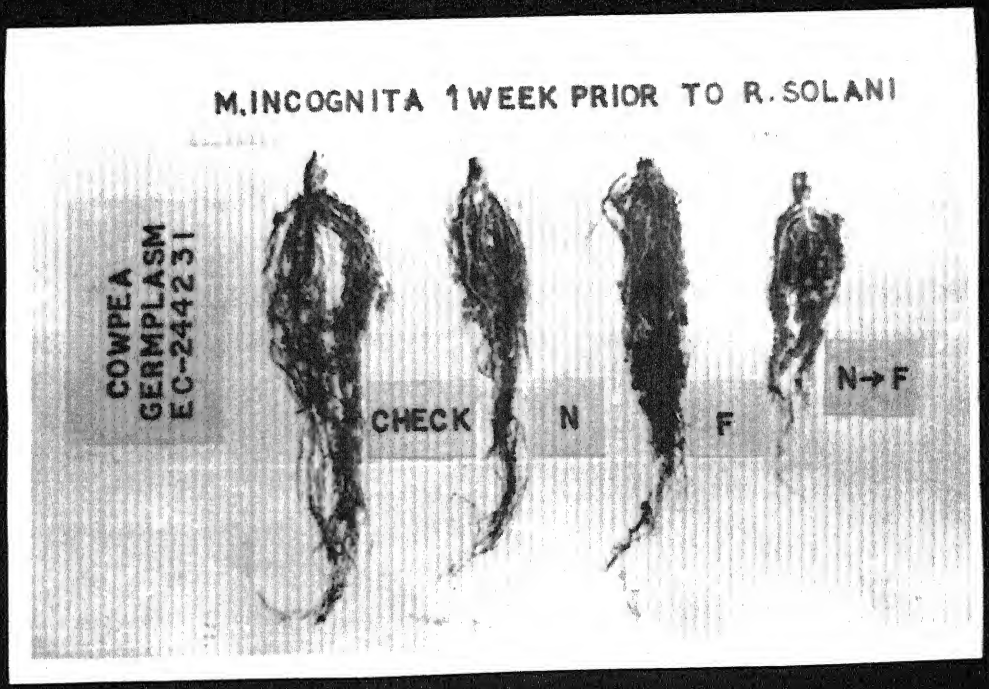

M.INCOGNITA 1WEEK PRIOR TO R.SOLANI
COWPEA
GERMPLASM
EC-244231
CHECK
N
F
N→F

FIG.7

Fig. 8: Root growth, nodulation, root-knot index when seedlings were inoculated with 1 g of R. solani 1 week prior to 1,000 larvae of M. incognita

CHECK = Uninoculated

N = Nematode alone

F = Fungus alone

F → N = Fungus prior to nematode

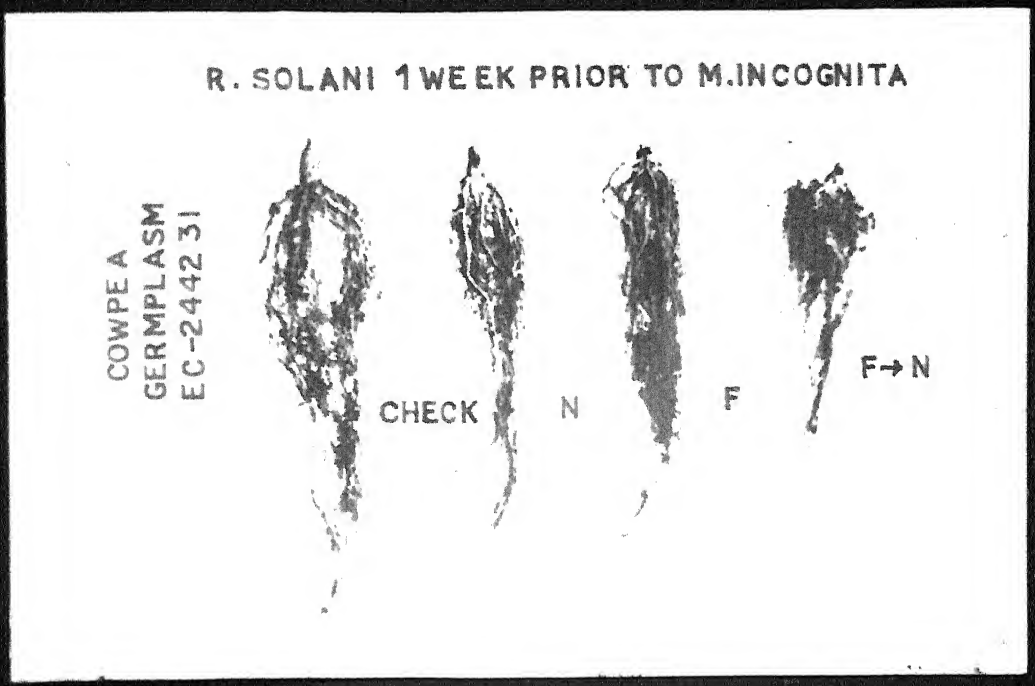

R. SOLANI 1 WEEK PRIOR TO M.INCOGNITA
COWPEA
GERMPLASM
EC-244231
CHECK
N
F
F→N

FIG. 8

observed that significant reduction occurred only in the treatment where the seedlings were inoculated with root-knot nematode and R. solani simultaneously. There was a significant decrease in nematode population in all the concomitant treatments as against the treatment where seedlings were inoculated with nematode alone.

Cowpea germplasm IL-163 (resistant to R. solani)
(Table 6; Figs. 9-12; Appendix IV)

The results given in table (6) show that when the seedlings of cowpea germplasm, IL-163 were inoculated with root-knot nematode alone or with fungus alone or with mixture of the two simultaneously or sequentially with nematode 1 week prior to fungus or fungus 1 week prior to nematode, the percent reduction in dry weight, 45 days after inoculation, over uninoculated one in the above treatments was 23.89, -0.38, 54.15, 29.87 and 25.19 g respectively; root-knot index was 2.90, 0, 2.20, 2.30 and 2.10 respectively and the final nematode population was 2559, 0, 2050, 2075 and 1742 respectively for the corresponding treatments.

Marked reduction in nodule index was also observed in all the treatments except where the seedlings were inoculated with fungus alone. The nodule index was 2.96, 3.33, 2.60, 2.86 and 2.86 against 3.40 in uninoculated plants for corresponding treatments. From the results presented above, it is clear that in all the treatments except where seedlings were inoculated with fungus alone, the reduction in dry weight

Table 6: Results of inoculating seedlings of cowpea germplasm, IL-163 (Resistant to R. solani) with 1,000 larvae of M. incognita and 1 g of R. solani singly and concomitantly (three different combinations) on plant growth, nodulation, root-knot index and nematode population, 45 days after inoculation

Treatment	Plant dry weight (g)		Nodule index	% reduction over uninoculated	Root-knot index	% reduction over nematode alone	Nematode population			R = Pf/Pi
	Root + shoot	% reduction over uninoculated					Per g of root	Per 200 g of soil	Total population	
Uninoculated	7.70	-	3.40	-	-	-	-	-	-	-
Nematode alone	5.86*	23.89	2.96*	12.94	2.90	-	1094	1465	2559	2.559
Fungus alone	7.73	-0.38	3.33	2.05	-	-	-	-	-	-
Nematode & fungus simultaneously	3.53*●	54.15	2.60*	23.52	2.20*	24.13	890	1160	2050	2.050*
Nematode 1 week prior to fungus	5.40*	29.87	2.86*	15.88	2.30*	20.68	780	1295	2075	2.075*
Fungus 1 week prior to nematode	5.76*	25.19	2.86*	15.88	2.10*	27.58	641	1101	1742	1.742*
C.D. at 5% level	0.85	-	0.29	-	0.31	-	-	-	-	0.244
C.D. at 1% level	1.20	-	0.41	-	0.43	-	-	-	-	0.342

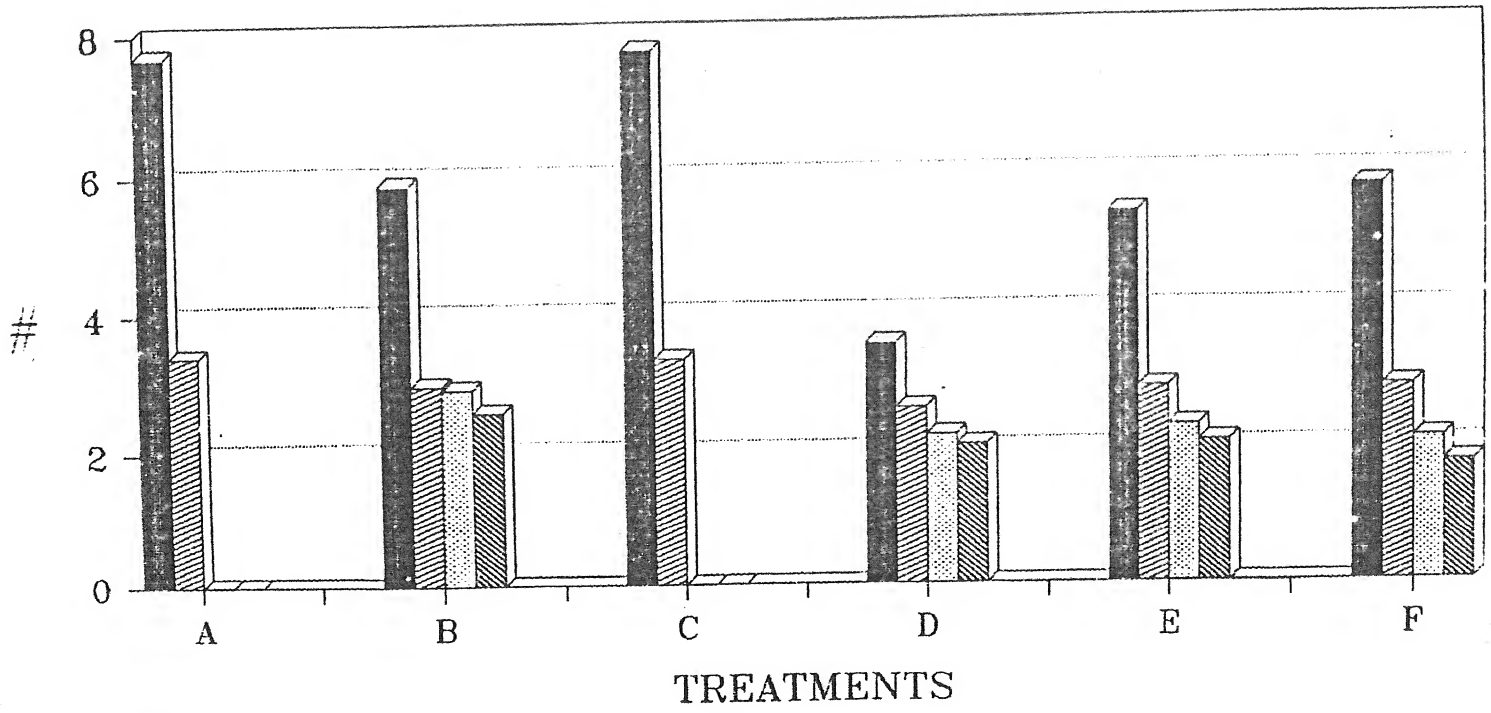
Each reading is an average of 3 replicates.

* Significant at 1% level against uninoculated.

● Significant at 1% level in between the concomitant treatments.

N.B. - For details please see Appendix IV.

Fig. 9: Plant dry weight, nodule index, root-knot index and nematode population when seedlings of cowpea germplasm IL-163 were inoculated with 1,000 larvae of M. incognita and 1 g of S. solani individually and concomitantly (three different combinations)
A = Uninoculated; B = Nematode alone;
C = Fungus alone; D = Nematode and fungus simultaneously; E = Nematode 1 week prior to fungus; F = Fungus 1 week prior to nematode.



DRY WEIGHT(g)/NODULE INDEX/ROOT-KNOT INDEX/NEMATODE POPULATION (X1000)

FIG. 9

Fig. 10: Plant growth, nodulation and root-knot index when seedlings were inoculated with 1,000 larvae of *M. incognita* and 1 g of *R. solani* simultaneously

CHECK = Uninoculated

N = Nematode alone

F = Fungus alone

N + F = Nematode and fungus simultaneously

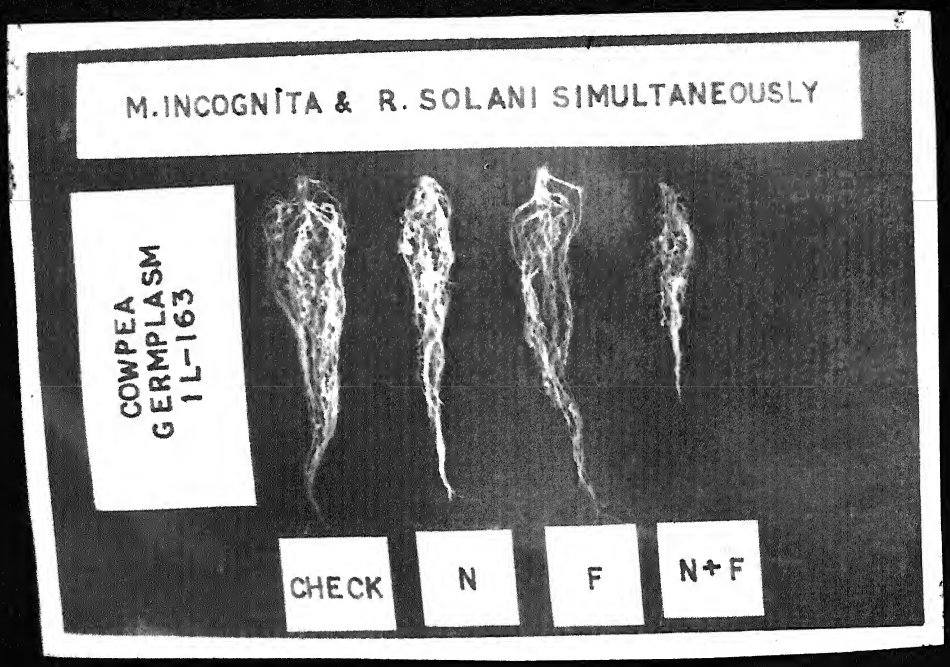

M. INCOGNITA & R. SOLANI SIMULTANEOUSLY
COWPEA GERMPLASM IL-163
CHECK
N
F
N+F

FIG. 10

Fig. 11: Root growth, nodulation and root-knot index when seedlings were inoculated with 1,000 larvae of *M. incognita* 1 week prior to 1 g of *R. solani*
CHECK = Uninoculated
N = Nematode alone
F = Fungus alone
N → F = Nematode prior to fungus

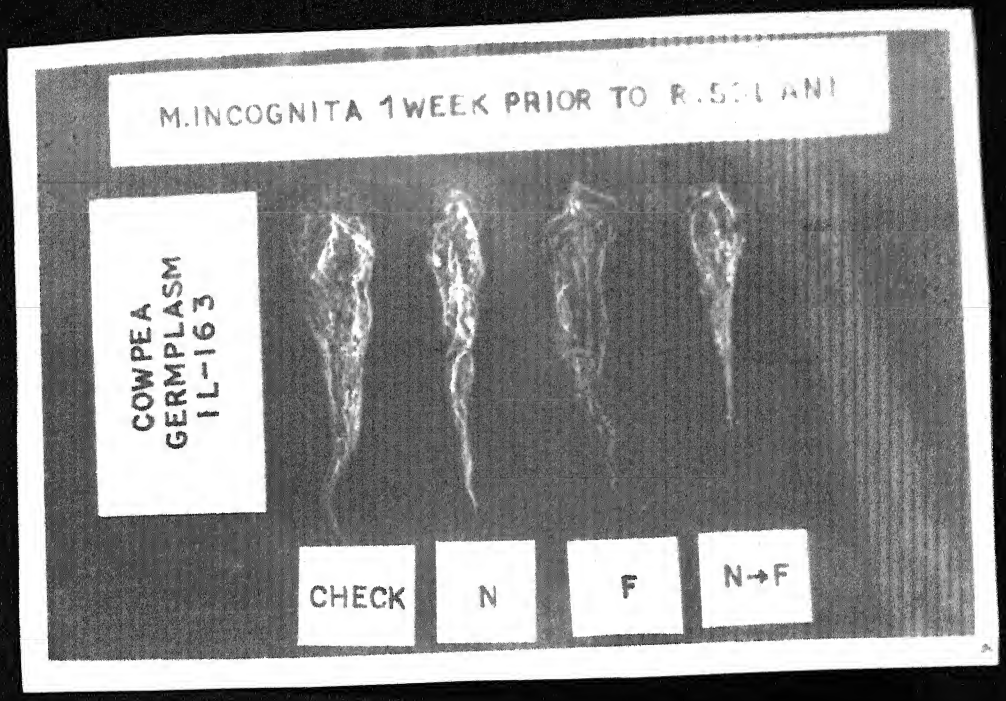

M.INCOGNITA 1WEEK PRIOR TO R.SOLANI
COWPEA
GERMPLASM
IL-163
CHECK
N
F
N→F

FIG. 11

Fig. 12: Root growth, nodulation, root-knot index when seedlings were inoculated with 1 g of *R. solani* 1 week prior to 1,000 larvae of *M. incognita*

CHECK = Uninoculated

N = Nematode alone

F = Fungus alone

F → N = Fungus prior to nematode

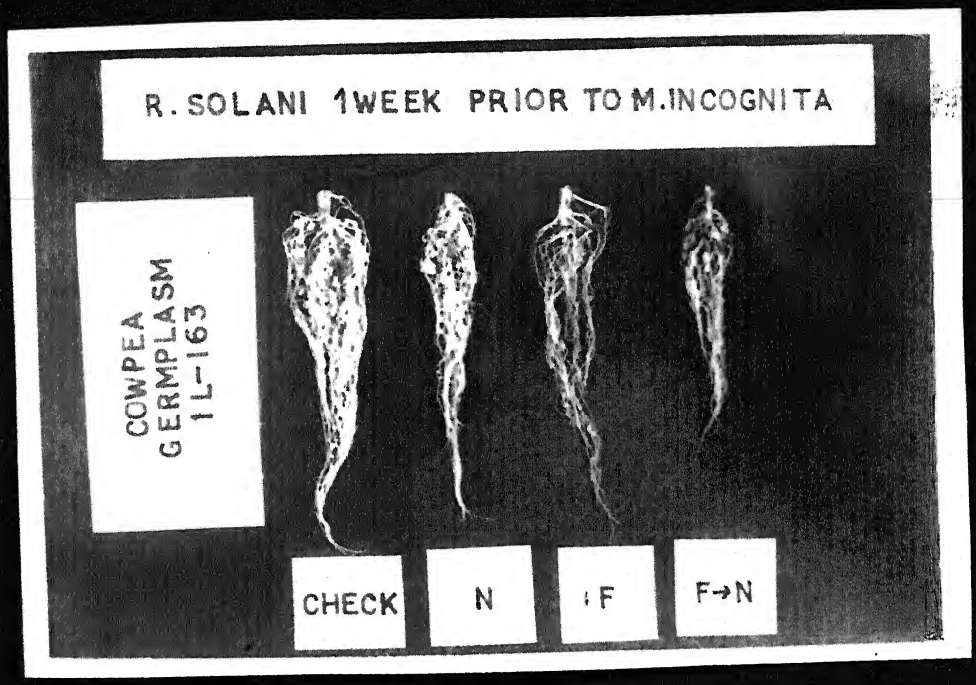

R. SOLANI 1WEEK PRIOR TO M.INCOGNITA
COWPEA GERMPLASM 1L-163
CHECK
N
F
F→N

FIG. 12

was significant (at 1% level) as against uninoculated and when C.D. at 1% level was calculated in between the concomitant treatments, it was observed that the significant reduction occurred only in the treatment where the seedlings were inoculated with nematode and fungus simultaneously. There was significant decrease in nematode population in all the concomitant treatments as against the treatment where the seedlings were inoculated with nematode alone.

The results obtained and presented in table (6) clearly show that the presence of nematode has broken the resistance of cowpea germplasm IL-163 against the fungus R. solani. It is interesting to note that the presence of nematode was not only able to break the resistance of cowpea germplasm IL-163 against fungus, but the damage was almost equal to that which was in fungus susceptible germplasm, EC-244231.

From the data given in table (5) and (6) it is clear that the optimal reduction in dry weight occurred where seedlings were inoculated with nematode and fungus simultaneously.

EFFECT OF INDIVIDUAL AND CONCOMITANT INOCULATION OF M. INCOGNITA AND R. SOLANI ON EMERGENCE/SURVIVAL OF SEEDLINGS

(Table 7; Fig. 13)

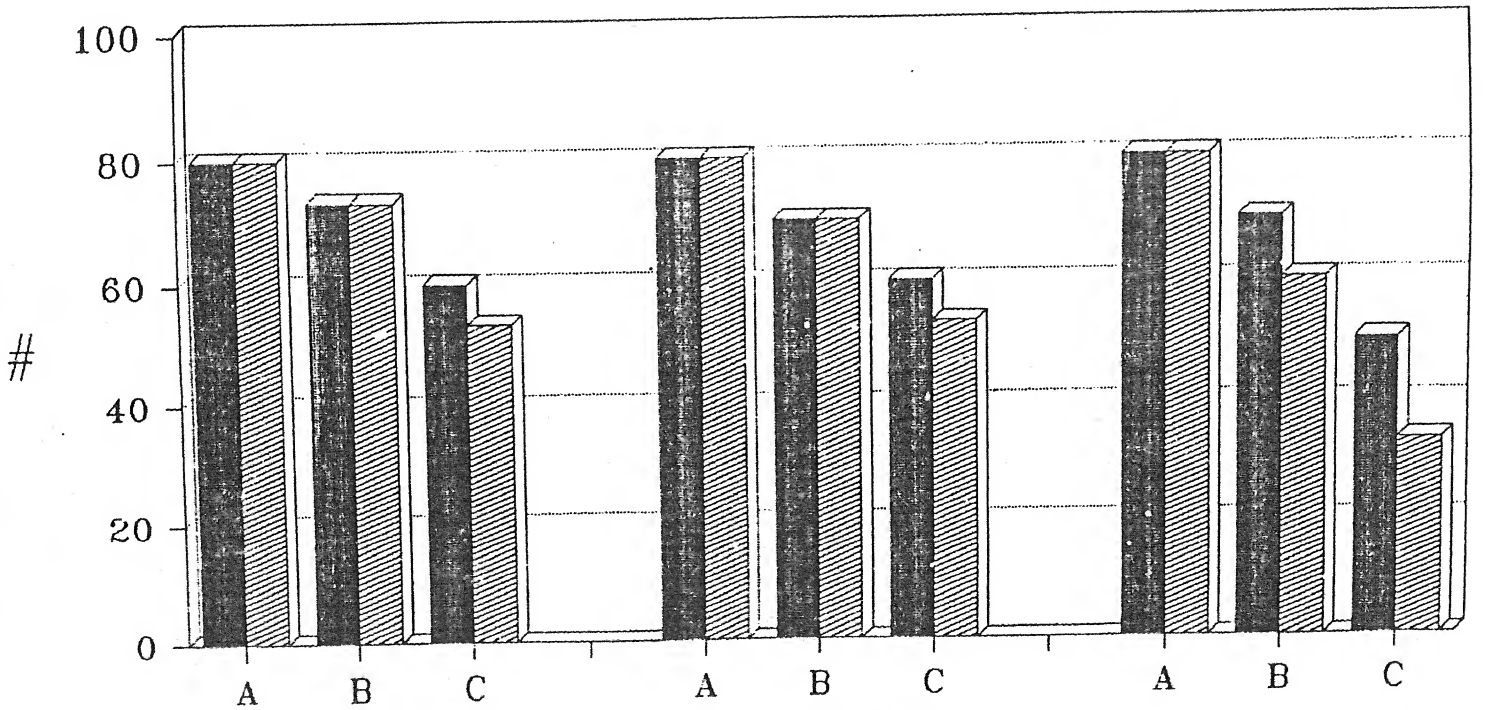
The results given in table (7) show that when seeds of cowpea germplasm, EC-244231 (susceptible to both

Table 7: Effect of individual and concomitant inoculation of *M. incognita* and *R. solani* on emergence/survival of seedlings of cowpea germplasm, EC-244231 (susceptible to both the pathogens)

Inoculum levels of *M. incognita*/kg soil	Percent emergence/survival of seedlings		
	Inoculum levels of *R. solani*/kg soil		
	0	1 g	5 g
Emergence of seedlings after one week			
0	80.0	73.3	60.0
1,000 larvae	80.0	70.0	60.0
5,000 larvae	80.0	70.0	50.0
Survival of seedlings after two weeks			
0	80.0	73.3	53.3
1,000 larvae	80.0	70.0	53.3
5,000 larvae	80.0	60.0	33.3

Each reading is an average of 3 replicates.

Fig. 13: Effect of individual and concomitant inoculation of *M. incognita* and *R. solani* on seedling emergence/survival of cowpea germplasm, EC-244231.



A=0g; B= 1g; C= 5g OF R. SOLANI

PERCENT EMERGENCE/SURVIVAL OF SEEDLINGS

FIG. 13

the pathogens) were sown in soil inoculated with 1,000 larvae and 5,000 larvae of M. incognita alone or with 1 g and 5 g of R. solani alone, the seedling emergence was 80.0, 80.0, 73.3 and 60.0 percent respectively and the survival of emerged seedlings was 80.0, 80.0, 73.3 and 53.3 percent against 80.0 percent in control (uninoculated soil). In soil inoculated concomitantly with nematode and fungus in different combinations i.e. 1000 lv. + 1 g fungus; 1000 lv. + 5 g fungus; 5000 lv. + 1 g fungus and 5000 lv. + 5 g fungus, the seedling emergence was 70.0, 60.0, 70.0 and 50.0 percent respectively and the survival of emerged seedlings was 70.0, 53.3, 60.0 and 33.3 percent respectively.

The above results clearly indicate that nematode alone had no adverse effect on emergence and survival of seedlings while the fungus alone has reduced the seedling emergence. The concomitant inoculation of the two pathogens had an adverse effect on the seedling emergence as well as on survival of seedlings. The maximum reduction occurred at higher inoculum levels of both the pathogens.

EFFECT OF SEED TREATMENT WITH NEMATICIDE (CARBOFURAN)/ FUNGICIDE (BAVISTIN) ON THE CONTROL OF DISEASE COMPLEX INVOLVING M. INCOGNITA AND R. SOLANI

(Table 8; Fig. 14; Appendix V)

The results presented in table (8) show that in uninoculated seedlings raised from seeds with no treatment, carbofuran treatment and bavistin treatment, the dry weight

Table 8: Effect of seed treatment with nematicide (Carbofuran)/fungicide (Bavistin) against disease development, nematode multiplication and plant growth, after 45 days of inoculation

Treatment	Dry weight (g)		Nodule index	% reduction over control	Root-knot index	Nematode population			
	Root + shoot	% reduction over control				Per g of root	Per 200 g of soil	Total population	R = Pf/Pi
1	2	3	4	5	6	7	8	9	10
Untreated uninoculated									
Control	11.06	-	3.86	-	-	-	-	-	-
Untreated inoculated									
Nematode alone	9.43	14.73	2.70	30.05	3.06	1652	1813	3465	3.465
Fungus alone	8.93	19.25	2.40	37.82	-	-	-	-	-
Nematode and fungus simultaneously	5.53	50.00	2.13	44.81	2.50	1037	1380	2417	2.41
Carbofuran treated uninoculated	10.99	0.63	3.33	13.73	-	-	-	-	-
Carbofuran treated inoculated									
Nematode alone	9.79	11.48	3.06	20.72	1.76	213	1333	1546	1.54
Fungus alone	9.73	12.02	2.60	32.64	-	-	-	-	-
Nematode and fungus simultaneously	7.43	32.82	2.66	31.08	1.80	235	1346	1581	1.581

contd.

1	2	3	4	5	6	7	8	9	10
Bavistin treated uninoculated	11.80	-6.69	3.56	7.77	-	-	-	-	-
Bavistin treated inoculated									
Nematode alone	10.00	9.58	2.50	35.23	2.16	283	1413	1696	1.696
Fungus alone	11.23	-1.53	2.83	26.68	-	-	-	-	-
Nematode and fungus simultaneously	8.13	26.49	2.83	26.68	1.56	316	1200	1516	1.516
C.D. at 5%	0.30	-	0.31	-	0.44	-	-	-	0.135
C.D. at 1%	0.42	-	0.44	-	0.62	-	-	-	0.189

Each reading is an average of 3 replicates.

N.B. - For details please see Appendix V

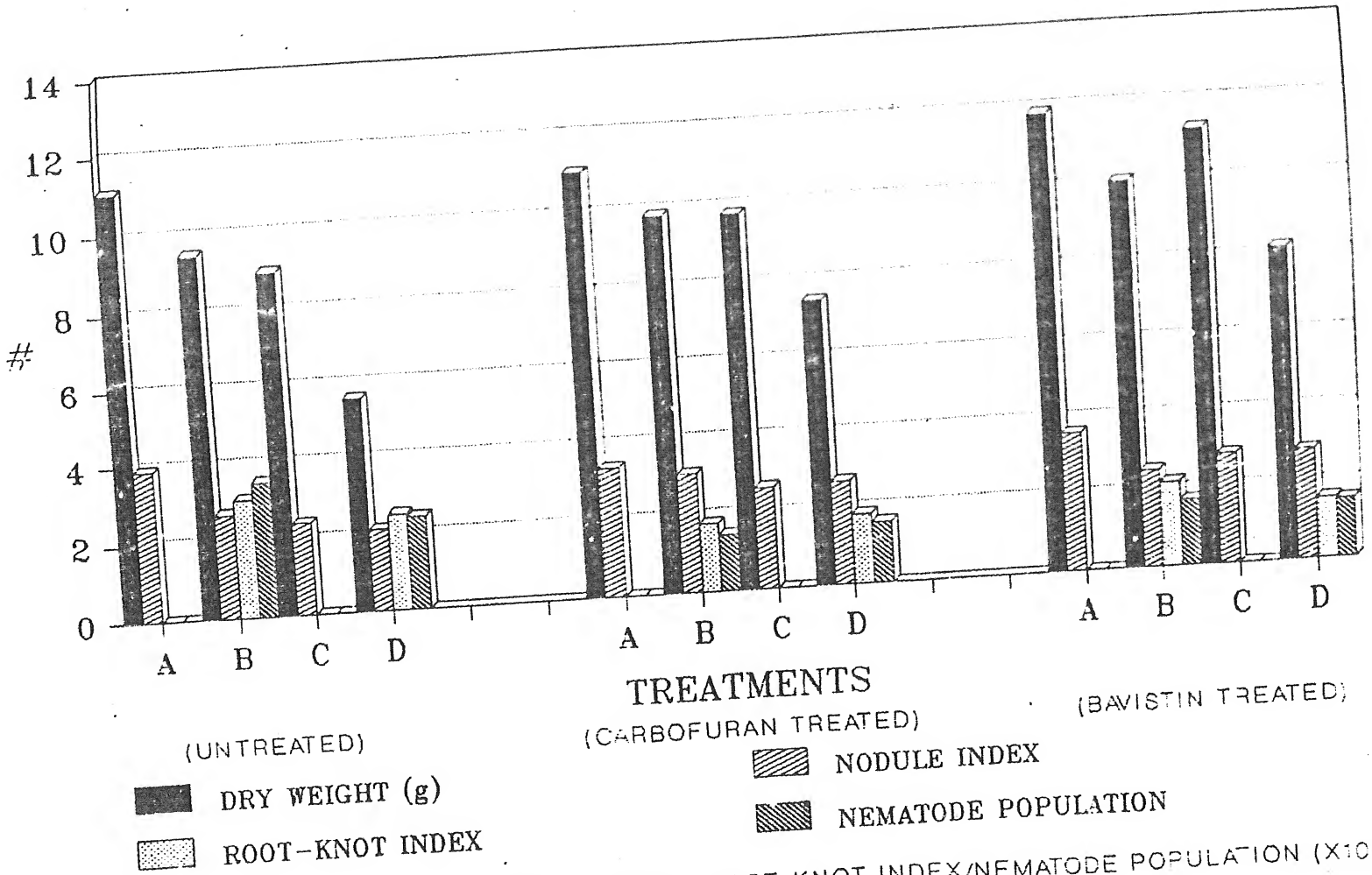
Fig. 14: Plant dry weight, nodule index, root-knot index and nematode population when seedlings raised from untreated, carbofuran treated and bavistin treated seeds of cowpea germplasm, EC-244231 were inoculated with 1,000 larvae of *M. incognita* and 1 g of *R. solani* individually and simultaneously

A = Uninoculated

B = Nematode alone

C = Fungus alone

D = Nematode and fungus simultaneously


14
12
10
8
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4
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0
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A B C D
A B C D
A B C D
TREATMENTS
(UNTREATED)
(CARBOFURAN TREATED)
(BAVISTIN TREATED)
DRY WEIGHT (g)
ROOT-KNOT INDEX
NODULE INDEX
NEMATODE POPULATION
DRY WEIGHT(g)/NODULE INDEX/ROOT-KNOT INDEX/NEMATODE POPULATION (X1000)

FIG. 14

of the plants, after 45 days, was 11.06, 10.99 and 11.80 g; nodule index was 3.86, 3.33 and 3.56 respectively.

Untreated seeds

When seedlings raised from untreated seeds were inoculated with nematode alone or with fungus alone or with nematode and fungus concomitantly, the percent reduction in dry weight of the plants, after 45 days of inoculation, over uninoculated one in the above treatments was 14.73, 19.25 and 50.00 respectively; the percent reduction in the nodule index was 30.05, 37.82 and 44.81 respectively. The reproduction factor of M. incognita when present alone or concomitantly with the fungus was 3.465 and 2.417 respectively.

Carbofuran treated seeds

When seedlings raised from carbofuran treated seeds were inoculated with nematode alone or with fungus alone or with nematode and fungus concomitantly, the percent reduction in dry weight of the plants, after 45 days of inoculation, over untreated uninoculated one in the above treatments was 11.48, 12.02 and 32.82 respectively; the percent reduction in nodule index was 20.72, 32.64 and 31.08 respectively. The reproduction factor of M. incognita when present alone or concomitantly with the fungus was 1.546 and 1.581 respectively.

Bavistin treated seeds

When seedlings raised from bavistin treated seeds were inoculated with nematode alone or with fungus alone or with nematode and fungus concomitantly, the percent reduction in dry weight of the plants, after 45 days of inoculation over untreated uninoculated one in the above treatments was 9.58, -1.53 and 26.49 respectively; the percent reduction in nodule index was 35.23, 26.68 and 26.68 respectively. The reproduction factor of M. incognita when present alone or concomitantly with the fungus was 1.696 and 1.516 respectively.

The above results clearly indicate that the seed treatment with carbofuran or bavistin improved the plant growth by reducing the adverse effect of individual and concomitant inoculation of M. incognita and R. solani. The nematode multiplication and root-knot index was also reduced.

DISCUSSION AND CONCLUSION

The role of plant parasitic nematodes in disease development is firmly established. They not only cause disease but they have also been reported to increase the disease severity if present with other types of micro-organisms. This is specially true in case of root diseases, since the roots are exposed to a variety of micro-organisms under field conditions. The literature pertaining to multi-pathogenic effects on plants have been reviewed by Pitcher (1963, 1965); Powell (1963, 1971a & b, 1979); Bergeson (1972) and Khan (1981).

In the present studies 100 exotic as well as Indian fodder type cowpea germplasms have been screened against M. incognita on the basis of percent penetration and nematode development within the roots of the germplasms. The different germplasms showed varied degree of resistance and susceptibility. Out of the 100 germplasms tested, 20 were proved to be highly resistant; 57 to be resistant; 17 to be susceptible and 06 to be highly susceptible (Table 1).

When all the above germplasms were screened against R. solani by blotter paper technique, the different germplasms showed different reactions to R. solani as measured on the basis of extent of damage caused to the root system. Out of the 100 germplasms, 14 were found to be

resistant; 29 to be moderately resistant; 41 to be tolerant; 11 to be moderately susceptible and 05 to be susceptible (Table 2).

Out of the above, two germplasms namely EC-244231 (susceptible to R. solani) and IL-163 (resistant to R. solani) have been selected for further studies keeping in view that these two are susceptible to M. incognita.

When seedlings of germplasm, EC-244231 were inoculated with four different inoculum levels i.e. 10, 100, 1,000 and 10,000 larvae of M. incognita, the plant dry weight decreased while the root-knot index and nematode population increased with an increase in inoculum levels. At low (10 lv.) inoculum level no material difference in plant dry weight has been observed (Table 3). Similar correlation with other nematodes including root-knot has also been observed by other workers (Swarup and Sharma, 1965; Oostenbrink, 1966; Siddiqui, 1969; Azam, 1975; Raut and Sethi, 1980).

On inoculation of the seedlings of germplasm, EC-244231 with four different inoculum levels i.e. 0.25, 0.50, 1.0 and 2.0 g of R. solani, the plant dry weight has been reduced with an increase in inoculum levels (Table 4). Similar correlation with R. solani on eggplant had been reported by Azam (1975).

Inoculation with root-knot nematode as well as with fungus caused reduction in nodule index. The reduced

nodulation in leguminous crops due to root-knot nematode has already been reported by Nigh (1966); Taha and Raski (1969); Balasubramanian (1971); Hussaini and Seshadri (1975); Sharma and Sethi (1976) and Ali et al. (1981). Similarly, reduced nodulation due to fungal attack has also been reported by Twng-Wah and Howard (1969) and Raut and Sethi (1981) in leguminous plants.

Increase in nematode population at high inoculum levels is understandable as the presence of nematode retards nodulation and in extreme cases galls are produced on nodules containing mature females. This fact is in accord with the findings of Nigh (1966); Taha and Raski (1969) and Jamal (1976).

Since little is known about the role of concomitant inoculation of the nematode and fungus on leguminous crops, therefore, this aspect was also undertaken. The role of concomitant inoculation of the two pathogens was studied on cowpea germplasm, EC-244231 (susceptible to the fungus R. solani) as well as on cowpea germplasm, IL-163 (resistant to the fungus R. solani).

When seedlings of germplasm, EC-244231 (susceptible to the fungus R. solani) were inoculated with root-knot nematode alone or with fungus alone or with the mixture of the both simultaneously or sequentially with nematode one week prior to fungus or fungus one week prior to nematode,

the significant reduction (at 1% level) in dry weight and nodule index occurred in all the treatments but the optimum reduction in dry weight and nodule index has occurred in the treatment where the nematode and fungus were inoculated simultaneously followed by nematode 1 week prior to fungus and then fungus 1 week prior to nematode, although the nematode alone and fungus alone was also able to cause significant reduction in dry weight and nodule index (Table 5).

Nematode population was also significantly reduced in the treatments where fungus and nematode have been inoculated simultaneously or nematode 1 week prior to fungus or fungus 1 week prior to nematode. The adverse effect of the fungus on nematode population may be due to toxic effect of R. solani (Alam et al., 1973; Azam et al., 1979) or due to damage to the roots depriving nematodes of feeding sites (Junaid and Khan, 1989) or due to nematoxic antimetabolites produced by the fungus (Sakhuja and Sethi, 1986).

When seedlings of germplasm, IL-163 (resistant to fungus R. solani) were inoculated with root-knot nematode alone or with fungus alone or with the mixture of the both simultaneously or sequentially with nematode 1 week prior to fungus or fungus 1 week prior to nematode, the reduction in dry weight and nodule index was significant at 1% level in

in all the treatments except in the treatment where the fungus alone was used (Table 6). Nematode population was also significantly reduced in the treatments of concomitant inoculations as compared to the nematode alone. This is due to the adverse effect of the fungus as already explained in the preceding paragraph.

It is interesting to note that the presence of nematode has broken the resistance of the germplasm, IL-163 against the fungus R. solani (Table 6). This may be due to the nematode attack which provides entry portals for the fungus and also cause physiological and biochemical changes in the host tissue which favour fungal attack and multiplication (Goswami et al., 1975; Nath et al., 1984). It is also observed that the presence of nematode has not only broken the resistance but the damage caused in the fungus resistant germplasm, IL-163 was almost equal to that which was in fungus susceptible germplasm, EC-244231 (Table 5 & 6). Similar type of results have been reported by Jenkins and Courson (1957); Thomason et al. (1959); Devis and Jenkins (1963) and Thakar et al. (1986) where they have observed that the presence of nematode has broken the resistance of the test plants against the fungus.

In both, fungus susceptible germplasm, EC-244231 and fungus resistant germplasm, IL-163, the optimum reduction in plant weight has occurred in the treatment where nematode and fungus were inoculated simultaneously (Table 5 & 6).

These findings with respect to optimum damage are in accord with those of Kawamura and Hirano (1967 & 1968); Michell and Powell (1972); Azam (1975); Chhabra *et al*. (1977); Reddy *et al*. (1979); Kumar *et al*. (1988) and Junaid and Khan (1989).

Although root-knot nematode, *M. incognita* and fungus *R. solani* individually had their adverse effect on the nodulation, this adverse effect on nodulation has been increased in all other treatments where the nematode and fungus were present together. This is in accord with the findings of Varshney *et al*.,(1987). The maximum reduction being in the treatment where both the pathogens were inoculated simultaneously. It is presumed that this reduction in nodulation may be due to the adverse effect on rhizobium by the excretions released from the root-knot nematode and *R. solani* infected roots (Rhode, 1972) or nutritional interference in the host plant (Masefield, 1958; Malek and Jenkins, 1964) or physiological changes in the host plant (Balasubramanian, 1971; Hussaini and Seshadri, 1975) or nematode attack may be responsible for early degeneration of the nodules and destruction of nodular tissue.

The nematode alone had no adverse effect on the emergence or survival of seedlings while the fungus alone is effective in reducing the seedling emergence. But the concomitant inoculation of both the pathogens cause an

adverse effect on the emergence as well as on the survival of the seedlings and the maximum reduction in emergence and survival has occurred at higher inoculum level of both the pathogens (Table 7).

The seed treatment with nematicide (carbofuran)/ fungicide (bavistin) significantly reduced the individual or combined adverse effect of the root-knot nematode, M. incognita and fungus R. solani. The nematode population was also reduced against the treatment where the untreated seedlings were inoculated with nematode alone (Table 8). It can be concluded that the carbofuran and bavistin improve the plant growth by reducing the individual or combined effect of M. incognita and R. solani. This is due to the nematicidal and fungicidal effect of carbofuran and bavistin. It is also observed that fungicide bavistin is more effective in reducing the adverse effect of the two pathogens as compared to carbofuran.

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* Not consulted in original.

APPENDICES

Appendix II: Effect of four different inoculum levels of R. solani on plant growth and nodulation, 45 days after inoculation of seedlings of cowpea germplasm, EC-244231 (susceptible to R. solani)

Treatment	Plant growth												Nodule index	% reduction over uninoculated
	Length (cm)				Fresh weight (g)				Dry weight (g)					
	Root	Shoot	Total	% reduction over uninoculated	Root	Shoot	Total	% reduction over uninoculated	Root	Shoot	Total	% reduction over uninoculated		
Uninoculated	24.13	57.33	81.46	-	8.00	24.80	32.80	-	1.70	5.16	6.86	-	3.80	-
0.25 g	22.56	55.46	78.02*	4.22	7.00*	24.00	31.00	5.48	1.40	4.80	6.20	9.62	3.50	7.89
0.50 g	20.56*	50.40*	70.96*	12.88	6.33*	23.46	29.79	9.17	1.10*	4.76	5.86	14.57	3.30	13.15
1.00 g	18.63*	43.63*	62.26*	23.56	4.96*	21.03*	26.15*	20.24	0.93*	3.96*	4.89*	28.71	2.80*	26.31
2.00 g	16.33*	40.16*	56.49*	30.65	4.10*	19.16*	23.26*	29.08	0.70*	3.30*	4.00*	41.69	2.50*	34.21
C.D. at 5%	1.46	1.82	2.04	-	0.59	1.86	2.56	-	0.23	0.63	0.76	-	0.49	-
C.D. at 1%	2.05	2.55	2.87	-	0.84	2.61	3.60	-	0.33	0.89	1.06	-	0.68	-

Each reading is an average of 3 replicates.

* Significant at 1% level against uninoculated.

IV: Results of inoculating seedlings of cowpea germplasm, IL-163 (resistant to R. solani) with 1,000 larvae of M. incognita and 1 g of R. solani singly and concomi
nodulation, root-knot index and nematode population, 45 days after inoculation

Plant growth															
Length (cm)				Fresh weight (g)				Dry weight (g)				Nodule index	% reduction over uninoculated	Root-knot index	% reducti over nematoc alone
Root	Shoot	Total	% reduction over uninoculated	Root	Shoot	Total	% reduction over uninoculated	Root	Shoot	Total	% reduction over uninoculated				
20.63	96.50	117.13	-	5.80	45.33	51.13	-	1.50	6.20	7.70	-	3.40	-	-	-
17.56	90.16	107.82	7.94	5.00*	41.83	46.83	8.40	1.10	4.76*	5.86*	23.89	2.96*	12.94	2.90	-
20.00	95.66	115.66	1.25	5.50	48.50	54.00	-5.61	1.50	6.23	7.73	-0.38	3.33	2.05	-	-
24.96*	92.13*	67.09*	42.72	2.90*	27.33*	30.23*	40.87	0.50*	3.03*	3.53*	54.15	2.60*	23.52	2.20*	24.13
15.30*	82.66*	77.96*	33.44	2.80*	32.33*	35.13*	31.29	0.80*	4.60*	5.40*	29.87	2.86*	15.88	2.30*	20.68
17.00	70.16*	87.16*	25.58	3.73*	38.50	42.23	17.40	0.86*	4.90	5.76*	25.19	2.86*	15.88	2.10*	27.58
2.96	6.75	6.97	-	0.48	6.12	6.34	-	0.30	0.70	0.85	-	0.29	-	0.31	-
4.16	9.48	9.79	-	0.68	8.59	8.90	-	0.42	0.99	1.20	-	0.41	-	0.43	-

ding is an average of three replicates.
ent at 1% level against uninoculated.